

ORIGINAL ARTICLE

EVALUATION OF ERYTHROCYTE GLUTATHIONE PEROXIDASE, SUPEROXIDE DISMUTASE AND TOTAL ANTIOXIDANTS IN CATARACT PATIENTS

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Abstract

Background-The pathogenesis of cataract has been found to be influenced by a number of factors including oxidative stress. Human body contains natural antioxidants, including the enzymes glutathione peroxidase and superoxide dismutase which help it withstand stress. Some environmental and nutritional factors can affect antioxidant systems.

Objective-This study was undertaken to assess the status of total antioxidants, glutathione peroxidase (GPx) and superoxide dismutase (SOD) in the Iranian population whose lifestyle is both nutritionally and environmentally different from other populations on which such studies have been conducted.

Methods-Forty-five patients with senile cataract and 35 controls were selected and matched. The levels of the above-mentioned enzymes and chemicals were measured in erythrocytes and plasma and compared by Student's *t*-test ($p < 0.05$ for significance).

Results-The level of GPx erythrocyte activity in patients and controls was found to be $51.24 \pm 13.46 \mu\text{g}_{\text{Hb}}$ and $40.87 \pm 7.04 \mu\text{g}_{\text{Hb}}$ respectively ($p < 0.0001$). SOD levels were $1239.83 \pm 275.96 \mu\text{g}_{\text{Hb}}$ in patients and $1126 \pm 201.72 \mu\text{g}_{\text{Hb}}$ in controls ($p = 0.045$). Total antioxidant status of the patients and controls were $1.39 \pm 0.27 \text{ mMol/L}$ and $1.64 \pm 0.36 \text{ mMol/L}$ respectively ($p < 0.001$).

Conclusion-Even though the relationships of these factors to cataractogenesis is still unknown, we believe that a better knowledge about their role could strengthen our understanding of the pathogenesis, diagnosis and perhaps the treatment of cataract. Still, it is safe to assume that educating people on consumption of a diet richer in antioxidants (like vegetables) is beneficial in preventing diseases like cataract.

Keywords • Oxidative stress • total antioxidants • cataract • glutathione peroxidase • superoxide dismutase

Introduction

Cataract is the single leading cause of blindness throughout the world. Over 50% of all cases of blindness can be attributed to cataract and more than 20 million people worldwide are affected.^{1,2} The disease is basically age-related and the number of people developing cataract is directly proportional to the increasing number of seniors in a global scale. In the developing countries, including India and

Kenya, blindness resulting from cataract evolves earlier in life and is three times more prevalent than in developed countries.³

Age, however, is not the only predisposing factor to cataract. Other factors such as hyperoxia, radiation, tryptophan deficiency, hypoproteinemia, diabetes, galactosemia, phenylketonuria and Wilson's disease have been implicated in its pathogenesis.⁴

While the mechanism of initiation and progression of cataract is being investigated, there is still no consolidated theory, which can predict the course or route of cataractogenesis. It is

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believed that oxidative mechanisms play an important role in the pathogenesis of cataract. Oxidative damage to the lenticular biomolecules is commonly seen in the majority of cases.

It is also thought that osmotic stresses and exogenous or endogenous oxidative stress play an important role in the pathogenesis of cataract.⁵ Oxidative stress may result from an imbalance between the production of reactive oxygen species (ROS) and the cellular antioxidant defence mechanisms. In the cells of the eyes, ROS may initiate a surge of toxic biochemical reactions such as peroxidation of membrane lipids and extensive damage to proteins causing intracellular protein aggregation and precipitation.^{6,7}

The human body has a variety of natural antioxidants, which help it withstand against oxidative insult. These systems scavenge free radicals and prevent oxidative damage. They include intracellular antioxidants such as the enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx).^{8,9} During the normal aging process and in inflammations, free radical production may increase and hence the defense mechanism may be depleted.

Few studies have been conducted on Iranians with senile cataract. Iranians have special environmental and nutritional patterns, which have a role in the oxidation process and in cataract formation. The aim of this study was to examine the levels of total antioxidants and the activity of erythrocyte GPx and SOD in Iranian patients with cataract.

Materials and Methods

Forty-five patients, diagnosed with senile cataract and 35 controls were selected and interviewed. Information was obtained about medical history, smoking habits, diseases such as diabetes, use of medication and occupational background. To prevent the possibility of statistical inefficiency, care was taken to match groups for age and sex, keeping in mind the conclusive association between age and frequency of cataract. Sex distribution of patients was 25 males (55%) and that of controls was 21 males (60%). Of the 45 patients, 23 were diagnosed with mixed cataract, 10 with nuclear cataract, 7 with cortical cataract and 5 with posterior subcapsular cataract (PSC). None of the patients in either group exhibited systemic diseases such as diabetes or hypertension. All subjects underwent a detailed ocular

examination by an ophthalmologist including external and slit-lamp examination, applanation tonometry and detailed funduscopy. Blood samples were obtained for complete blood count, erythrocyte sedimentation rate and blood chemical analysis (including FBS and BUN).

Total hemoglobin was measured by the colorimetric method (Cat. No.10-532, Zeist Chemi, Iran). Total antioxidant status was measured at 37°C in the Cobas Mira S-plus Automated Analyzer (Roche Analytical Instruments, New Jersey, USA) based on the method derived from the observation that when 2,2'-azinobis 3-ethylbenzothiazolin-6-sulfonic acid (ABTS) is incubated with a peroxidase (metmyoglobin) and H₂O₂, the long-lived radical cation ABTS will be formed.¹⁰ In the presence of antioxidant reductants and hydrogen donors, the absorbency of this radical cation is quenched to an extent related to the antioxidant capacity of the added fluid.

Erythrocyte GPx was measured by the method reported by Paglia and Valentine.¹¹ GPx catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP⁺. Erythrocyte SOD activity was measured using xanthine and xanthine oxidases to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride (INT).¹² Both methods were adopted for Cobas Mira S-plus Automated Analyzer. All materials were prepared by Randox Laboratories (Crumlin, UK).

The data were expressed as means±SD by using SPSS/PC software. Comparison between the patient and control groups was made using student's *t*-test.

Results

The results are summarized in Table 1. Mean age (±SD) of patients and controls were 68.4±7.7 and 66.3±6.6 respectively (p=0.207) showing no significant difference and indicating a properly matched group.

Evaluation of the mean erythrocyte GPx activity for both groups indicated a mean value of 51.23±13.46 μ/g_{Hb} for patients and 40.866±7.04 μ/g_{Hb} for controls (p=0.0001).

Erythrocyte activity of SOD in patients and controls was found to be 1239.83±275.96 μ/g_{Hb}

Table 1. Comparison of mean age, glutathione peroxidase, superoxide dismutase and total antioxidant status in patients and controls.

Parameter	Patients (n=45)	Controls (n=35)
Age (\pm SD) (years)	68.4 \pm 7.9	66.3 \pm 6.6*
GPx (\pm SD) (μ /g _{Hb})	51.24 \pm 13.46	40.87 \pm 7.04**
SOD (\pm SD) (μ /g _{Hb})	1239.83 \pm 275.96	1126 \pm 201.72†
Total antioxidant status (\pm SD) (mMol/L) of plasma	1.39 \pm 0.27	1.64 \pm 0.36‡

*p=0.207, **p<0.0001, †p=0.045, ‡p<0.001.

and 1126.80 \pm 201.72 μ /g_{Hb} respectively (p=0.045). SOD activity of nuclear cataract in the patients and controls was 1312.45 \pm 253.71 (μ /g_{Hb}) and 1126.80 \pm 201.72(μ /g_{Hb}) respectively.

Total antioxidant status was 1.39 \pm 0.27 mMol/L in patients and 1.64 \pm 0.36 mMol/L in controls (p=0.001), indicating a significant difference between controls and patients.

Discussion

Oxidative stress, diabetes and lack of certain essential nutrients may be multiple risk factors in cataract which cause a loss in transparency and impairment of lens metabolism in the course of this degenerative disease.

We have conducted a small scale study on the Iranian population who might have a notably different nutritional and environmental lifestyle than that of populations in the other parts of the world,^{13,14} and disturbances in both enzymatic and non-enzymatic components of the antioxidant defense systems were examined. Even though some contradictory evidences exist, many claim that changes in antioxidants are an index based on the activity of erythrocyte enzymes such as GPx and SOD, plasma antioxidant status and certain blood biochemical variables have a potential influence in the pathogenesis of this disease (research in progress).^{15,16} The results of our study confirm some previous findings which correlate with human cataract.

Doubling of erythrocyte GPx activity was present in patients with cataract compared to that of controls. Delcourt¹⁵, studying the same enzyme in a larger population sample, reported a similar result. This rise in activity of GPx may be considered as a risk factor in cataract patients. The same author showed a 6-fold and a 2-fold increase in the enzyme activity of cortical and nuclear cataract, respectively. Girodon¹⁷ reported an

increase in GPx activity in persons receiving dietary supplement of minerals only.

Also in this study, the erythrocyte SOD showed more than a one-fold increase in activity, demonstrating the highest levels for nuclear cataract in patients compared to controls. Delcourt's patients exhibited the same pattern of activity. Jacques¹⁶ who studied the relationship between the biochemical markers of antioxidants in 112 subjects, did not report any statistically significant higher levels of erythrocyte GPx and SOD activity. He proposed that there may be a synergistic effect of these enzymes and antioxidant vitamins such as vitamins C and E; the postulation being that the high level of one vitamin with a low level of one of the antioxidant enzymes may provide adequate protection and reduce oxidant damage. However, a shortcoming in Jacques' experiment is that it examined nutrient status after pathology was already visible.

The status of plasma total antioxidants which include the chain breaking antioxidants such as ascorbate, urate and bilirubin and the membrane preventive antioxidants such as β -carotene and vitamin E, was lower compared to these levels in controls given in table which indicates an increased risk for cataract formation. Other researchers, including Jacques¹⁶, Mohan¹³, and Knekt¹⁸ reported the same result. Low levels of such extra-cellular and membrane antioxidants make the cells and tissues susceptible to different types of oxidation. The body's defence mechanism activates the increase in synthesis of enzymes such as GPx and SOD thereby combating the effects of this oxidation process.

With the understanding that these relationships to cataract formation are still unknown¹⁹ and that there is a limited ability to measure antioxidant levels directly (*in vivo*) in the lens, our results must be interpreted cautiously. Still, it is safe to assume that the most practical means of delaying cataract

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formation is to educate and increase the awareness of large populations about the benefits of consuming a diet rich in antioxidants (like vegetables). On the other hand evaluation of these biochemical parameters could provide a tool and a marker for the prevention or early detection of cataract.

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