INTRODUCTION

Cataract is clouding or opacity of the eye lens, which is the main cause of blindness. A recent report by the World Health Organization (WHO) showed that 47.8 percent of blindness cases are caused by cataract. Seventeen percent (20.5 million) of Americans over 40 years are suffering from cataract and it may increase to more than 30 million by 2020. The exact statistics are not available regarding the prevalence of cataract in Iran, but it seems that one-fifth of the population over 40 years are suffering from cataract in the country.

Cataract is a multifactorial disease. Risk factors, such as aging, high blood glucose levels, exposure to ultraviolet radiation, alcohol consumption, metabolic disorders, genetic factors, oxidative stress, and trauma are involved in cataract creation. Although, age is not the only factor for cataract, but this disease is mainly related to age. The research results showed that 82% of the population over the age of 50 years have cataract.

Epidemiologic and experimental studies showed that oxidative stress is the main cause of initiation and progression of senile cataract. Reactive oxygen species (ROS), which basically include molecules such as superoxide anion, hydrogen peroxide, and hydroxyl radicals were detoxified by enzymes such as glutathione peroxidase, superoxide dismutase, and catalase. In this way, the antioxidant system and the ROS remain in a state of homeostasis. When oxidative stress is induced by some external or internal factors, the homeostasis is disturbed and ROS will denatured many essential intracellular molecules such as nucleic acids, proteins, and lipids.

As such, this study was done to evaluate the oxidative stress level and the antioxidant activity of serum GPx and SOD in patients with cataract.

METHODOLOGY

This is a case-control study conducted on 153 people who were referred to the Imam Ali Hospital with non-randomised sampling from 2014 to 2015. Of these, 74 patients, who were suffered from cataract with the confirmation of ophthalmologist, were stayed in the case group; and 79 patients, who were referred to the annual eye check-ups and had no cataract, stayed in the control group. The patients were matched in terms of age and gender. Cataract diagnosis was performed by an ophthalmologist under slit-lamp examination and other eye tests including Goldmann applanation tonometer, fundoscopy, retinoscopy, keratometry, etc. were done in...
order to rule out other eye disorders. All those people had impaired vision and their visual acuity was less than 3.10, and they had senile cataract at least in one of their eyes. They had no other ocular disorders that can justify reduction of their vision. Patients with secondary cataract as a result of diabetes, steroid use, and trauma were excluded from the research. The control group was in good health status and that was confirmed with their medical history in filling the related questionnaire and physiological experiments, and laboratory tests such as cholesterol, triglycerides, blood sugar, blood pressure and absence of disease related to the oxidative stress. None of the subjects have history of cardiovascular disease, liver disease, gastrointestinal or renal disorders. History of smoking, alcohol consumption, and external hormones intake was not observed in them.

The participants in the study were asked not to consume any vitamin supplements or carotenoid until the initiation of the experiments; whereas, tea and coffee intakes were limited in them. Their blood samples were collected for biochemical tests.

Venous blood (3 ml) was phlebotomised under sterile condition by disposable syringes after 12 hours of fasting. Samples were centrifuged for 15 seconds with temperature at 3500 rpm. Then, serum was separated and transferred to the clean tubes and stored in the freezing temperature of -70°C.

Measuring the enzymes activity is based on the spectrophotometric evaluation. Other tests and biochemical parameters such as blood glucose, triglycerides, cholesterol, etc. were measured using routine clinical biochemistry tests.

To assess the balance between oxidant and anti-oxidant (PAB), TMB (3,3',5,5' Tetramethylbenzidine) and cation were used as a redox indicator (due to the electrochemical and optical properties). In this method, the oxidant and anti-oxidant balance was simultaneously measured in an experiment by two different reactions. In an enzymatic reaction, chromogenic TMB (color-causing) was oxidised by peroxidoxin to colored cation and in a chemical reaction, the TMB cation becomes colorless combination by antioxidants. Photometric absorption was compared with certain absorption of a series of standard solutions having different ratios (0-100%) of hydrogen peroxide and uric acid mixture. 7,8

The measurement of the superoxide dismutase enzyme activity was performed using Ransod-Randox kit. Half ml of isolated RBC was washed 4 times with 3 ml of saline and then they were centrifuged for 10 minutes in the centrifuge device with the rate of 3000 rpm. The washed RBC was mixed with 2 ml of cold distilled water and kept at 4°C for 15 minutes. Obtained lysate was used to determine the SOD activity.

Measurement of the GPx enzyme activity was performed using Ransel-Randox kit. 0.5 ml of isolated RBC were diluted by 1ml of diluents and incubated for 5 minutes. This diluted substance was mixed with Drabkin’s reagent and used to measure GPx.

Data are presented as mean ± SD or median (IQR), and frequencies with percentages to report qualitative variables. All experimental data in this study were statistically analysed with SPSS version 11.5. The normality of data checked by Kolmogorov-Smirnov test. The normal data evaluated by Student’s t-test, and others were evaluated by Mann-Whitney test. Results were considered significant at p <0.05.

**RESULTS**

Basic demographical and clinical characteristics of cataract and healthy groups were presented in Table I. Of the total 74 cases, 43 (58%) patients were females and 31 (42%) were males; and 79 individuals were in the control group, of which 45 (57%) were females and 34 (43%) were males. The median age (years) of patients was 65 (19) in the case study group; and was 63 (9) years in the control group. The studied people were matched in terms of age and gender. Cholesterol, triglycerides, glucose, systolic blood pressure, and diastolic blood pressure were measured. Among these factors, the cholesterol and triglycerides serum levels and diastolic blood pressure between two groups showed a significant difference (p<0.0001).

Table II compared prooxidant-antioxidant balance (PAB) in serum of cataract and non-cataract subjects. PAB was measured in an experiment by two different reactions. In an enzymatic reaction, chromogenic TMB (color-causing) was oxidised by peroxidoxin to colored cation and in a chemical reaction, the TMB cation becomes colorless combination by antioxidants. Photometric absorption was compared with certain absorption of a series of standard solutions having different ratios (0-100%) of hydrogen peroxide and uric acid mixture. 7,8

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49.01 ±20.90 in control group, and it was significantly increased in cataract patients (74.66 ±27.91 U/ml, p<0.001). Also, the GPx levels were 283.7 (102.3 U/ml) in control group and it was significantly increased in cataract patients 444.4 U/ml 322.4, p=0.018). The similar pattern also shown about SOD levels (U/ml); so, it was higher in cataract group than the control group, and its difference was significant (p<0.001, Table II).

DISCUSSION
The present study shows that oxidative stress, SOD and GPx levels significantly increased in serum of cataract patients. Until now, many studies have been done to evaluate the role of oxidative stress in the pathogenesis of age-related cataract. The results of measuring the activity of GPx and SOD in cataract patients are various in different researches. Delcourt and colleagues examined the relationship between cataract and serum anti-oxidants. Their results showed the similar pattern like this study for SOD and GPx levels in patients compared to healthy controls. Also, the erythrocyte SOD and GPx activity increased in one study, directed by Mohammadi, et al., also showed that the activities of SOD, GPx and Catalase (CAT) in cataract group were lower than those in the control group and the oxidative stress products malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), conjugated diene (CD), advanced oxidation protein products (AOPP), protein carbonyl (PC), and 8-hydroxydeoxyguanosine (8-OHdG) were significantly increased in serum in cataract patients. Chandrasena and colleagues also showed that SOD and GPx activity in patients with cataract decreased compared to healthy controls.

Many researchers have postulated that diminished antioxidant activity in addition to elevated levels of free radicals plays a pivotal role in cataractogenesis in senile age group. According to a study conducted by Kuar and colleagues on 50 patients of 45-75 years with cataract and 50 healthy subjects, demonstrated that lipid peroxidation products (MDA) in the serum of patients with cataract is higher than in healthy controls. SOD and GPx activity in patients with cataract decreased compared to healthy controls. Chang et al., also showed that the activities of SOD, GPx and Catalase (CAT) in cataract group were lower than those in the control group and the oxidative stress products malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), conjugated diene (CD), advanced oxidation protein products (AOPP), protein carbonyl (PC), and 8-hydroxydeoxyguanosine (8-OHdG) were significantly increased in serum in cataract patients. Chandrasena and colleagues also showed that SOD and GPx activity in patients with cataract decreased compared to healthy controls.

The controversy shown in different studies may be for some reasons. Studies indicate that natural antioxidants can act as prooxidants, which produce free radicals and cause DNA damage and mutagenesis. For deletion of free radicals, different parts of antioxidants system including vitamins, antioxidants enzymes, and their cofactors are needed. If every antioxidant act, it can produce prooxidant that will be omitted by next antioxidant cycle. Thus, a lack of each cycle can increase the prooxidants and the result will be oxidative stress. Because all antioxidants cycles cannot be evaluated, it seems that measuring total oxidative stress will be more effective.

Oxidative stress is a term used to describe any challenge in which prooxidants predominate over antioxidants. It may be due to either increased production of ROS or decreased levels of antioxidants (enzymatic and non-enzymatic or both). Oxidative stress is thought to play a crucial role in the development of age related cataract. Mechanistically, oxidative stress leads to increased production of ROS which in turn causes increased production of \( H_2O_2 \) in aqueous humour as well as in lens. \( H_2O_2 \) is several folds higher in aqueous humor of cataract patients. ROS and \( H_2O_2 \) damage proteins and nucleic acids and also can oxidize the sulphydryl groups. The redox setpoint changes rapidly upon initiation of oxidative stress and irreversible oxidative damage quickly develops.

The lens defence system constitutes enzymatic antioxidants, that is, SOD, CAT, and GPx utilising superoxide and \( H_2O_2 \). However, the SOD and GPx activity increased in current study, but it was shown in some other references that protective systems decrease with the age and long-term exposure to oxidative stress predispose lens cells at risk for cumulative oxidative damage and cataract formation. Higher systemic oxidative stress increases the risk of developing age-related cataract. The effectiveness of SOD and other antioxidant enzymes is limited to several reasons such as their deactivation with aging. Being macromolecules, they cannot penetrate the certain sensitive sites of oxidation in nucleic acids and in proteins. The lens is surrounded by aqueous and the vitreous humors, fluids which lack the enzymatic defences. Therefore, the lens cell membranes, which are continuously exposed to a photochemical oxidative environment due to the continued light penetration during the long periods of photopic vision, remain susceptible to photo damage.

Although this research was carefully carried out, but, we are still aware of its limitations and shortcomings. First of all, the research was conducted in senile cataract only. Second, the sample size was small.

CONCLUSION
Oxidative stress is in the foreground of cataract formation. Although aging itself can play a role in generating oxidative stress, but our results clearly indicate oxidative imbalance is more pronounced. It seems the increased production of SOD and GPx enzymes cannot compensate this imbalance. Further studies should be conducted to elucidate the molecular mechanisms by which antioxidants modulate their protective role, in order to identify potential pathways; and more importantly, new protective factor.

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REFERENCES


