Serum levels of SOD, MDA and glutathione peroxidase in different subtypes of cataract patients

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Abstract

Background: Oxidative stress is essentially an imbalance between the production of free radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants. Since the reactive oxygen species (ROS) generated from multiple sources is capable of producing deleterious effects. Body has its own defence mechanism against the oxidative damage by ROS. These defence strategies act in the form of Antioxidants.

Methods: The present study was carried out in the Department of Biochemistry, Govt. Medical College, Amritsar in collaboration with Department of Ophthalmology, Ram Lal Eye Hospital, Amritsar attached to Govt. Medical College, Amritsar. 50 patients suffering from cataract attending the OPD and wards of Ramlal Eye Hospital diagnosed on the basis of history, clinical signs/symptoms and slit lamp examination were selected for the study. 50 age matched normal healthy individuals from the same population were selected to serve as controls. The comparison was done by students 't' test on the number of variables for each parameter.

Result: Our results confirm that oxidative stress is responsible for the development of all three subtypes of cataract. The use of antioxidants may be helpful to prevent or delay cataractogenesis.

Conclusion: We found that there is a significant disequilibrium status of antioxidant system in serum in various types of age-related cataract patients compared with the control group. The activities of SOD, GPx in cataract group were lower than those in the control group. The oxidative stress products MDA were significantly increased in serum of cataract patients.

Key words: Oxidative stress, Cortical cataract, Posterior sub- capsular, Nuclear cataract

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Introduction

Oxidative stress is thought to play a crucial role in the development of age related cataract.[1] Cataract is a clouding of the lens in the eye leading to a decrease in vision. Cataracts are most commonly due to aging but may also occur due to trauma, radiation exposure, be present from birth or occur following eye surgery. In developing countries like India, the magnitude of the problem is over whelming. Age related cataract is the most common variety of cataract. Usually some degree of cataract is present after the age of 50 years.[2,3]

Glutathione function and metabolism

Glutathione (γ-L-glutamyl-L- cysteinylglycine) is the most abundant low molecular weight thiol in the cells of all aerobic organisms. In the lens, most of the free glutathione is maintained in the reduced form (GSH), and all of the enzymes and pathways that are involved in the synthesis and degradation of glutathione in the lens have been identified.[4,5,6] The reduction of oxidized glutathione to reduced form is accomplished by the enzymes glutathione reductase. The concentration of glutathione is not uniform across the lens. It is high in the cortical regions and is relatively lower in the nucleus The highest concentration of glutathione is found in the epithelial layer. In the normal human lens the level of glutathione in the cortex is not dependent on age.[7]

Antioxidant defense mechanisms of the lens

In the normal human eye it is estimated that H_2O_2 exists in the aqueous humor at concentration of ~ 30μm. Much higher values have sometimes been found in the aqueous humor of cataractous patients.[8]

Research done in cultured lens epithelial cells has shown that enzymes in the glutathione redox cycle, as well as the enzyme catalase are needed in the detoxification of H_2O_2.[9]

An important pathway for protecting the lens from oxidative damage and for detoxification of xenobiotics is the mercapturic acid pathway with the lead enzyme glutathione – S –transferase.

The cataractous lens

Cataract is usually defined as any opacity in the lens. The possible causes, mechanisms, biochemical and biophysical changes occurring during cataractogenesis have been the subject of intense study for many years.[10,11] The three main types of age

dependent cataract are cortical, nuclear and cupuliform.[12]

Cortical cataract

Cortical cataracts form in the shell layer of the lens and gradually extends from the outside of the lens to the centre. These are caused by uptake of water into the lens after the breakdown of lens fibers into smaller particles. This process increases the osmotic pressure within the lens, thereby causing the aqueous to flow through the capsule into the lens substance. The Cuneiform opacity is the most characteristic form of age dependent cataract.

Nuclear cataract

Nuclear cataract is an exaggeration of the physiologic sclerotic change that results in loss of vision. Sclerosis is considered to be a conserving process since it resists disintegration. Most individuals 65 years of age or older develop some degree of nuclear sclerosis vision usually remains relatively unaffected. With increasing sclerosis, the color of the nucleus at the slit lamp progresses from yellow to orange, to brown and in extreme cases to black.

Cupuliform cataract

Cupuliform cataract is a common form of senile opacification and usually occurs at an earlier age than cortical or nuclear cataract. It consists of a thin layer of granules located just beneath the capsule, usually in the posterior cortical layer. It often has a discrete margin which gives it a cup shape, hence the name Cupuliform Cataract, or Posterior Subcapsular Cataract.

Material and Method

The study was carried out on 100 subjects of either sex with the age ranging from 40-70 years. Out of these 50 were Cataract patients in age group 40-70 years, attending the Out patient department (OPD) and wards of Opthalmology Department, Ram Lal Eye Hospital attached to Government Medical College, Amritsar. Fifty adult patients of either sex suffering from cataract were selected for study. It was case-control prospective study. The patients and the controls screened for serum superoxide dismutase (SOD) malondialdehyde (MDA), and glutathione peroxidase.

Collection and processing of blood samples

10 ml of venous blood was taken from the patients of Cataract and healthy controls in dry disposable syringe under aspetic conditions by vein puncture in antecubital vein, in a sterile, dry acid washed vial for biochemical analysis. 2 ml blood sample was collected in heparinized vial for estimation of whole blood glutathione peroxidase. 4 ml of blood was allowed to stand for half an hour. After the clot formation, the blood sample was centrifuged at 3000 rpm for 10 minutes. After that supernatant (serum) was taken for biochemical investigations. These biochemical assays were done on the same day.

Following investigations were carried out in all the patients and controls by given methods:

Investigations

1. Serum superoxide dismutase (SOD) was analyzed by applying the method of Marklund and Marklund (1974) (modified by Nandi and Chatterjea, 1988).[13]
2. Serum malondialdehyde (MDA) was estimated by applying the method of Satoh (1978).[14]
3. Glutathione Peroxidase was estimated by a method of Paglia and Valentine.[15]

Results

The present study was carried out in the Department of Biochemistry, Govt. Medical College, Amritsar in collaboration with Department of Ophthalmology, Ram Lal Eye Hospital, Amritsar attached to Govt. Medical College, Amritsar. 50 patients suffering from cataract attending the OPD and wards of Ramlal Eye Hospital diagnosed on the basis of history, clinical signs/symptoms and slit lamp examination were selected for the study. 50 age matched normal healthy individuals from the same population were selected to serve as controls. The comparison was done by students ‘t’ test on the number of variables for each parameter.

Table 1 shows age-wise distribution of the subjects who were divided into three groups, group I included subjects in the age range of 40-50 years and there are 10 patients in this group. Group II included subjects in the age range of 51-60 years and there were 19 patients in this group. Group III included subjects in the age range of 61-70 years and there were 21 patients in this group.

Table 1: Age wise distribution of the subjects

Group	Age (in years)	Controls	Patients
I	40-50	3	10
II	51-60	18	19
III	61-70	29	21

Table 2 shows the distribution of the control and patients sex wise. There were 24 males and 26 females in the control group. There were 28 males and 22 females in the patients. It was seen that males were male in number as compared to females in the patients group.

Table 2: Sex wise distribution of the subjects

Subject	Control	Patients
Male	24	28
Female	26	22

Table 3 shows mean serum MDA levels in cataract patients according to the type of cataract they develop. The mean±SD in MDA levels in the patients who had cortical cataract was 5.14±1.75. In case of nuclear cataract the mean levels were 5.77±1.58 nmol/ml. In case of posterior subcapsular cataract the levels were 5.54±1.72 nmol/ml. The highest levels were seen in patients of nuclear cataract.

Table 3: Mean serum mda levels in cataract patients according to type of cataract

Type of cataract	Mean (nmol/ml)	S.D	S.E
Cortical (n=20)	5.14	1.75	0.39
Nuclear (n=10)	5.77	1.58	0.5
Posterior subcapsular (n=20)	5.54	1.72	0.38

Table 4 shows mean serum SOD levels in cataract patients according to the type of cataract they develop. The mean±SD in SOD levels in the patients who had cortical cataract was 2.65±0.36 u/ml. In case of nuclear cataract the mean levels were 2.74±0.47 u/ml. In case of posterior subcapsular cataract the levels were 2.87±0.38 u/ml. The levels were highest in patients with posterior subcapsular type of cataract.

Table 4: Mean serum sod levels in cataract patients according to type of cataract

Type of cataract	Mean (u/ml)	S.D	S.E
Cortical (n=20)	2.65	0.36	0.08
Nuclear (n=10)	2.74	0.47	0.14
Posterior subcapsular (n=20)	2.87	0.38	0.08

Table 5 shows the mean blood glutathione perioxidase levels in cataract patients according to the type of cataract they developed. The level in case of patients with cortical cataract were 28.66±11.17, while the levels in patients with nuclear cataract were 24.68±13.03. In case of patients with posterior subcapsular cataract the levels were 24.56±12.23. The mean levels were highest in the patients who had cortical cataract.

Table 5: Mean serum glutathione peroxidase levels in cataract patients according to type of cataract

Type of cataract	Mean (u/gm Hb)	S.D	S.E
Cortical (n=20)	28.66	11.17	2.49
Nuclear (n=10)	24.68	13.03	4.12
Posterior subcapsular (n=20)	24.56	12.23	2.73

Discussion

Cataract is the leading cause of blindness, accounting for 50% of blindness worldwide. The ocular lens, which is continually exposed to light and ambient oxygen, is at high risk of photoxidative damage resulting in cataract. Oxygen free radicals appears to impair not only lens crystallins but also proteolytic enzymes whose function it would be to eliminate the damaged proteins.[16]

When clouding of the lens impairs vision, a clinically significant cataract is present. Currently there is no medical treatment for age related cataract. The only treatment is surgical removal of the affected lens when vision is sufficiently impaired. A major goal of past and on going epidemiologic studies is the identification of risk factors for cataract. So that strategies for the prevention of cataract can be developed.[17]

Experimental evidence suggests that free radicals accumulation lead to oxidative stress which plays role in the pathogenesis of cataract aand can be prevented by antioxidants. In addition, a recent study found that cataract patients tended to have lower serum levels of vitamin C, E or A as compare to control subjects.[18]

Senile cataract indicates the opacity of ocular lenses occurring in old and especially in very old people. Lens proteins are extremely long living and often show oxidative damages.

A new scientific base is emerging to support a protective role of fruits and vegetables rich with antioxidants in cataract formation. The evidence reviewed provides additional support for increased consumption of a wide variety of vegetables in particular dark green leafy and deep yellow, orange ones and a wide variety of fruits, in particular, citrus and deep yellow orange ones.[19]

Peroxidation of lipids produces free radicals like hydrogen peroxide, superoxide anion and hydroxyl radicals, resulting in structural alteration of membrane with release of cell and organelle contents, loss of essential fatty acids with formation of cytosolic aldehyde and peroxide products. Malendialdehyde is major end product of free radical reaction on membrane fatty acids. All these ROS are potentially dangerous to tissues.

In this study, a significant increase in serum MDA level ($p<0.001$) was observed in patients compared to

controls (Table 3). Increase in MDA level observed could be due to increased oxidative stress (age related) or decrease in antioxidant defense mechanism and vice-versa. In case of development of age related cataract, LPO may also be the real cause of destruction of the plasma membrane of the lenticular fibres and the subsequent oligmerization of the crystalline lens.[20] Significant decrease in serum SOD level ($p<0.001$) was observed in patients compared to controls (Table 4) that were similar to findings of other investigators.[21] SOD in enzymatic antioxidant while provides first line of the defense that acts by quenching O_2 and converting it into H_2O_2. There may be two reasons for lowering of SOD:

1. As more and more ROS like O_2 are produced SOD is being used up in the process when it converted O_2 to H_2O_2.
2. H_2O_2 also causes inhibition of SOD activity. There are several classes of SOD that differ in their metal bending ability, distribution in different cell compartments and sensitivity to various reagents.

$$O^{\circ}_2{}^{-} + O^{\circ}_2{}^{-} + 2H^{+} \xrightarrow{SOD} H_2O_2 + O_2$$

The present study also indicates age related decrease in glutathione peroxidase activity. The difference in the levels among controls and patients was statistically highly significant ($p<0.001$)

The observation were similar to findings of other investigators.[22,20]

The GPx scavenges the highly reactive lipid hydroperoxide in the aqueous phase of cell membrane.[23] The GSH antioxidant system, is the body's Power house for diffusing and disposing of radicals that threaten the cell, tissue and organ damage, thus slowing the approach of age. During aging, the lens loses its antioxidants potencies such as many been seen with the decrease of glutathione or the expression of anti-oxidant enzymes.[24] Among the antioxidant enzymes, the important enzymes are superoxide dismutase, catalase, glutathione peroxidase and Glutathione S-transferase.[25]

In the present study the serum MDA levels in males and female groups of controls and patients were compared. The difference between the levels of MDA in males and females was statistically insignificant ($p>0.05$).

Serum SOD levels in males and females groups of controls and patients were compared. The difference between the level of SOD in males and females was statistically insignificant ($p>0.05$).

Whole blood glutathione peroxidase levels in males and females groups under study were compared. The difference between the levels in males and females was statistically insignificant ($p>0.05$).

Table 3 compares the mean serum MDA levels in cataract patients according to type of cataract. There were 20 cases of cortical cataract, 10 cases of nuclear cataract and 20 cases of posterior subcapsular cataract. The mean serum MDA levels in cortical cataract cases was 5.14 nmol/ml±1.75. In case of nuclear cataract the levels were 5.77±1.58 nmol/ml while in case of posterior sub capsular cataract the levels were 5.54±1.72 nmol/ml. The highest levels were seen in patients of nuclear cataract.

Table 4 compares the serum SOD levels in cataract patients according to type of cataract. The mean serum SOD levels in cortical cataract cases were 2.65±0.36 u/ml. In case of nuclear cataract the mean serum SOD levels were 2.74±0.47 u/ml. While in case of posterior subcapsular cataract cases the levels were 2.87±0.38 u/ml. The levels were highest in patients with posterior subcapsular type of cataract.

Table 5 compares the mean blood glutathione peroxidase levels among three subtypes of age related cataract. The levels in case of patients with cortical cataract were 28.66±11.17 u/gm Hb. While in nuclear cataract the levels were 24.68±13.08 u/gm Hb. The levels were 24.56±12.23 u/gm Hb in posterior subcapsular cataract cases.

Conclusion

In conclusion, the present data indicates that oxidative stress as measured by pro-oxidant and antioxidant levels in blood may play an important role in age related cataract development. Antioxidant therapy may have a role to play in delaying the onset and progression of age related cataract. Antioxidants may be useful for prophylaxis or therapy against cataract. There is a limited ability to measure antioxidant levels directly (in vivo) in the lens, Therefore our results must be interpreted cautiously. It is safe to assume that the most practical means of delaying cataract formation is to educate and increase the awareness of large population about the benefits of consuming a diet rich in antioxidants (like vegetables and fresh fruits).

Evaluation of three parameters could provide a tool and a marker for the prevention or early detection of cataract act.

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