PRO-OXIDANT AND ANTI-OXIDANT STATUS IN SENILE CATARACT PATIENTS

Satya Devi Uppu*, Madhur M. Gupta

Dept. of Biochemistry, NKP Salve Institute of Medical Sciences and Research Centre, Digdoh Hills, Hingna, Nagpur 440 019, Maharashtra, India

Corresponding author: Dr Satya Devi Uppu

ABSTRACT:
Senile cataract is a multifactorial disease, but oxidative stress is considered to be most important risk factor. The aim of our study is to assess the levels of prooxidants by measuring the lipid peroxidation products in the form of the Malondialdehyde (MDA) and the antioxidant enzyme levels like Superoxide dismutase (SOD), Glutathione peroxidase (GPX) in the blood. A case control study was carried out in total number of 50 newly diagnosed cataract cases and 50 age matched controls. MDA, a marker of oxidative stress antioxidant enzymes Glutathione peroxidase (GPX) and Superoxide dismutase (SOD) were measured by enzymatic spectrophotometric method. Significantly increased levels of serum lipid peroxides in the form of MDA (P<0.001) were observed in cataract patients when compared to controls. Significantly decreased levels of SOD, GPX were observed in cataract patients as compared to normal healthy control (p<0.001). In the present study, it was concluded that oxidative stress plays an important role in the onset of senile cataract.

Key words: Glutathione peroxidase, Malondialdehyde, Reactive oxygen species, Senile cataract, Superoxide dismutase.

INTRODUCTION:
Senile cataract is one of the major cause of preventable blindness affecting about 50% of the blindness cases worldwide. Any opacity in the lens or its capsule whether developmental or acquired is called cataract. A senile cataract is the presence of lens opacity (excluding early cortical changes) which could be ascribed to congenital secondary or other specific cause with visual acuity of 6/9 or worse. The etiopathogenesis of senile cataract is multifactorial, and it has yet to be fully understood. However imbalance between the prooxidant and antioxidant levels thought to play major role in the pathogenesis of cataract [1].

Prooxidants are chemicals that induce oxidative stress either by generating Reactive Oxygen species (ROS) or by inhibiting antioxidant system. The loss of transparency occurs because of abnormalities of the lens protein and consequent disorganization of the lens fibre [2]. Antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation reaction can produce free radicals which inturn start chain reactions, that lead to damage or death to the cell.
Antioxidants terminate these intermediates and inhibit other oxidation reactions. Inhibition or insufficient levels of antioxidants cause oxidative stress and may damage or kill cells [3].

The lipid peroxidation represents the oxidative tissue damage which is caused by hydrogen peroxide, the superoxide anion and the hydroxyl radicals which results in the structural alteration of the membrane, with the release of the cell and organelle contents and the loss of the Essential Fatty Acid, with the formation of cytosolic aldehyde and peroxide products. MDA is the major end product of free radical reaction on the membrane fatty acid [4]. Renugarg [5], Bhuyan et al [6] who investigated Lipid peroxidation (LPO) as one of the possible mechanisms of cataractogenesis in the humans found that MDA, major product of lipid peroxides was increased in cataract patients than controls. Babhizhayer [7] reported a strong correlation between the degree of opacity of the lens and concentration of LPO products in tissues.

SOD is an enzymatic antioxidant which provides defence that acts by quenching oxygen and converts it into hydrogen peroxide. This protects cell membrane from damage which is caused by ROS. But decreased SOD may lead to increased lipid peroxides results in cellular rigidity and deformability [8]. An altered activity of SOD in the cataract patients has been revealed recently [9]. GPX scavenges the highly reactive lipid hydro peroxide in the aqueous phase of the cell membrane. During aging, the lens loses its antioxidant potencies such as may be seen with the decrease of GPX or the expression of antioxidant enzymes [10].

Earlier studies showed that the oxidative stress which is caused due to accumulation of free radicals plays a role in the pathogenesis of cataract and that this process can be prevented or ameliorated by antioxidants. The present study was aimed at determining the concentration of lipid peroxidation product in the serum such as MDA and antioxidant enzyme activity by estimating SOD, GPX and their role in the pathogenesis of senile cataract.

**MATERIALS AND METHODS**

The present study consisted of fifty patients with cataract and fifty normal healthy controls, who were in the age group of 45-70 years, presented to the outpatient department of Ophthalmology, NKP Salve Institute of Medical Sciences and Research Centre, Nagpur. Inclusion criteria was diagnosed cases of cataract and exclusion criteria includes history of Diabetes mellitus, hypertension, refractive errors, any other systemic illness and use of any antioxidant or vitamin supplementations. Before the start of the study, the approval of Institutional ethics committee was obtained. Written informed consent was obtained from all 100 subjects. 5 ml of venous blood samples were collected in EDTA bottles and plain bulbs from patients with cataract and healthy individuals.

Blood samples were centrifuged at 3000 rpm for 10 minutes. MDA was measured by the method of randox laboratory. This method was based on the fact that lipid peroxide condense with 1-methyl 2-phenyl indole(MPI) under acidic conditions resulting in formation of a red chromophore. To determine specifically lipid peroxide in plasma, proteins are precipitated to remove water soluble MPI reactive substance. The level of lipid peroxide is expressed in terms of malondialdehyde. Tetra methoxypropane, which is converted quantitatively to MDA, was used as standard. The erythrocytic GPX was estimated by spectro photometric randox kit
method [11], the principle being that GPX catalyses oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH the oxidized glutathione is immediately converted to reduced form with a concomitant oxidation of NADPH to NADP. The decrease in absorbance was measured at 340 nm. SOD was measured from hemolysate by spectrophotometric enzymatic kit method [12]. The principle employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2- (4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The statistical analysis was done by student t-test.

RESULTS AND DISCUSSION

Serum lipid peroxide concentration in the form of MDA was significantly increased in the cataract patients (p<0.001) as compared with controls. The level of antioxidant enzyme SOD was significantly decreased in cataract patients (p<0.001) compared to that in the controls. The blood levels of GPX were decreased in the cataract patients (p<0.001) compared to those in the control group (Table1).

Table1: Levels of MDA, SOD, GPX in Senile cataract patients and healthy subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control n=50</th>
<th>Cases n=50</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA nmol/ml</td>
<td>1.986 ± 0.74</td>
<td>2.7 ±0.62</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SOD Unit/L</td>
<td>215.66± 44.04</td>
<td>114.7± 28.31</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>GPX Unit/ml</td>
<td>5499.24±674.1</td>
<td>3535.9±836.1</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

n= Number of cases or control groups        All values are expressed in mean ± SD

Human senile-cataract is a multi-factorial disease but oxidative stress plays an important role in the pathogenesis of cataract. As a result of oxidative stress, a series of highly reactive intermediates are produced like hydrogenperoxide, superoxide anion radical, hydroxy radicals and they can react with proteins, nucleic acids, lipids and carbohydrate of cell leading to peroxidative damage to biological membranes resulting in cataract. Lipid peroxides formed are unstable compounds they tend to degrade rapidly to variety of subproducts. MDA is used as a marker of lipid peroxidation.

In our study, the levels of MDA were significantly increased in cataract cases compared to controls. The observations of present study are in agreement with previous studies Garg et al [5], Donma et al [4], Babizhayer et al [7], Indranil et al [13]. As age advances there is increased peroxidative damage to lenticular membranes by oxygen free radicals in eye fluids and tissues. Thus, the continuing oxidation damage to lens results in increased levels of MDA in cataract patients.
To protect from ROS in the lens like other tissues, contain a series of defense mechanisms called as antioxidants. In the present study, the enzymatic antioxidant status was studied by estimating serum SOD and GPX activity. SOD levels were decreased when compared to controls (p<0.001). SOD is an enzymatic antioxidant which provides first line of the defense that acts by quenching superoxide radical and converting it into H$_2$O$_2$ [14]. Reasons for lowering of SOD in our study may be as more and more ROS like superoxides are produced, SOD is being used up in the process when it converts superoxide radical to H$_2$O$_2$ which also causes inhibition of SOD activity.

Our findings are same as Garg et al [5], Donma et al [4], Fecondo, Augustyn [15]. There are also reports where there is no significant change or even increased SOD levels in blood of cataract patients.GPX is a compound which defends the lens against oxidative insults, being directly involved in reducing the disulphides, being a pivotal cofactor in the detoxification of H$_2$O$_2$ and acting as a free radical quencher. In the present study, GPX levels are decreased when compared to the controls. Our findings in GPX follow similar trend as Nourmahamadi et al [16]. However, in contrast to these data, the increased levels of antioxidant enzymes have been reported to be associated with cataract [17, 18].

Our study reveals that the pro-oxidants predominate over anti-oxidants in senile cataract patients. As age advances, cataract becomes a common disorder. Prooxidant and antioxidant imbalance plays a key role in aging which was proved by many studies. It is strongly implicated that oxidative mechanisms play a major role in pathogenesis of cataract as concluded by many previous studies. The antioxidant enzyme activity levels reflected the change which took place in the development of senile cataract. Anti-oxidant therapy may have a role to play in delaying the onset of senile cataract.

REFERENCES