Brief Communication: Erythrocyte Antioxidant Enzymes in Patients with Cataract

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Abstract. The pathogenesis of cataract has been found to be influenced by a number of factors including oxidative stress. Catalase, glutathione peroxidase (GPX), and superoxide dismutase (SOD) are some of the antioxidant enzymes that protect the body from oxidative damage. The present study investigates the activities of erythrocyte catalase, GPX, and SOD with respect to senile cataract (non-diabetic cataract) and osmotic cataract (diabetic cataract) in a Sri Lankan population. One hundred and two non-diabetic subjects (50 with cataract and 52 non-cataract) and 106 diabetic subjects (56 with cataract and 50 non-cataract) were recruited into the study. Erythrocyte catalase, GPX, and SOD activities were assayed and the data were analysed by t-test (p <0.05 for significance). In the non-diabetic group, significantly low levels of catalase, GPX, and SOD activities were associated with cataract when compared with non-cataract. No significant changes in catalase, GPX, and SOD activities were observed in the diabetic group between cataract and non-cataract. Senile cataract (non-diabetic cataract) was associated with significantly low levels of erythrocyte catalase, GPX, and SOD when compared with non-cataract. No significant changes in catalase, GPX, and SOD activities were observed in the diabetic group between cataract and non-cataract. Senile cataract (non-diabetic cataract) was associated with significantly low levels of erythrocyte catalase, GPX, and SOD when compared with non-cataract. Positive correlations were observed between catalase and SOD (r = 0.75), catalase and GPX (r = 0.63), and SOD and GPX (r = 0.59) in subjects with senile cataracts. Our results indicate that erythrocyte antioxidant enzyme levels are decreased in senile cataract as opposed to osmotic cataract. Assays of these erythrocyte enzyme activities could provide a marker to identify individuals predisposed to senile cataract.

Keywords: oxidative stress, antioxidants; cataract, catalase, glutathione peroxidase, superoxide dismutase

Introduction

Cataract is one of the leading causes of blindness in the world today. Over 50% of all cases of blindness can be attributed to cataract and more than 20 million people worldwide are affected [1,2]. Cataract occurs in diabetic subjects (osmotic cataract) as well as in non-diabetic subjects (senile cataract); in the latter group, age and radiation effects are the main predisposing factors.

Free radicals and reactive oxygen intermediates (ROI) have been implicated in a wide variety of degenerative diseases including cataractogenesis [3]. It is believed that oxidative stress and osmotic stress are involved in the pathogenesis of cataract [4]. Oxidative stress may result when the cellular antioxidant defense mechanisms are unable to keep pace with the detoxification of ROI. These ROI mediate peroxidation of membrane lipids and cause extensive damage to proteins, leading to irreversible deleterious effects [5]. Osmotic stress may lead to the development of cataract in diabetic individuals. This is due to the accumulation of an osmotically active sugar, sorbitol, in the lens tissue [6].
Antioxidant enzymes that intercept and inactivate ROI are synthesized by all aerobic organisms [7]. Catalase, GPX, and SOD are important antioxidant enzymes that detoxify oxygen free radicals and hydrogen peroxide and thereby prevent oxidative damage. The normal aging process is thought to occur mainly as a result of degeneration of enzymes that are involved in the antioxidant defense mechanism [8]. Reduced glutathione (GSH) plays a major role in the regulation of the redox status of the cell and protects tissues from lipid peroxidation [9].

Studies on the antioxidant status of the lens and blood in cataract patients have been extensively reported. However, very few studies have been conducted on Sri Lankan patients with cataract. In developing countries, including Sri Lanka, India, and Kenya, cataract evolves earlier in life and is 3 times more prevalent than in developed countries [8]. The specific environmental and nutritional patterns of Sri Lankans may have a role in the oxidation process and in cataract formation. The present study investigated the levels of erythrocyte catalase, GPX, and SOD activity with respect to cataract in a diabetic group and a non-diabetic group of Sri Lankans.

Materials and Methods

Ethical clearance for the study was obtained from the Ethics Committee of the Faculty of Medicine, University of Kelaniya, Sri Lanka. One hundred and two non-diabetic subjects and 106 diabetic subjects in the age group 40-70 yr were recruited to the study from the Diabetic and Ophthalmological Clinics of the North Colombo Teaching Hospital, Sri Lanka. Information was obtained regarding medical history and occupational background and informed consent was obtained from all subjects. A complete ophthalmological examination including slit lamp examination was done on all subjects by an ophthalmologist. Of the non-diabetic subjects, 50 were diagnosed as cataract (21 females, 29 males, age 57 ± 9 yr) and 52 as non-cataract (27 females, 25 males, age 52 ± 10 yr). Of the diabetic group, 56 were diagnosed as cataract (24 females, 32 males, age 61 ± 9 yr) and 50 as non-cataract (20 females, 30 males, age 54 ± 8 yr). Three ml of venous blood was obtained from every subject under aseptic conditions using disposable syringes and needles. Spectrophotometric assays of erythrocyte catalase, GPX, and SOD activities were performed within 3 hr of collection of the blood samples, using reagent kits manufactured by Randox Labs, Ltd., (Crumlin, UK): Ransel-Randox for GPX and Ransod-Randox for SOD; catalase was measured by the method of Goth [10].

For SOD assays, 0.5 ml of separated RBCs was washed 4 times with 3 ml of 0.9% saline, centrifuging for 10 min at 3,000 rpm after each wash. Washed RBCs were mixed with 2 ml of cold distilled water and kept at 4°C for 15 min. The lysate was used to determine the SOD activity. For erythrocyte GPX assays, 0.5 ml of separated RBCs was diluted with 1 ml of diluting agent and incubated for 5 min. The diluent was mixed with double strength Drabkin`s reagent and used for GPX assays. For erythrocyte catalase assays, 0.02 ml of separated RBC`s was diluted with 4 ml of phosphate buffer (pH 7.4), and 0.1 ml of saponin solution (20 g/L) was added. Then, 0.03 ml of the lysate was mixed with hydrogen peroxide and ammonium molybdate and the absorbance of the final sample was measured at 405 nm against a reagent blank [10].

Data were expressed as mean ± SD; differences between enzyme activity levels in population groups were tested using the t-test, assuming equal variances in the two samples under comparison.

Results

In the non-diabetic group, significant decreases in the activities of erythrocyte catalase, GPX, and

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Non-diabetic subjects</th>
<th>Diabetic subjects</th>
</tr>
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<tbody>
<tr>
<td>Cataract (Non-cataract)</td>
<td>118.1 ± 11.7a</td>
<td>115.5 ± 8.9a</td>
</tr>
<tr>
<td>Cataract (senile)</td>
<td>104.7 ± 7.8a</td>
<td>115.4 ± 7.7x</td>
</tr>
<tr>
<td>GPX (U/ml x 10³)</td>
<td>6.97 ± 1.75b</td>
<td>5.40 ± 1.60c</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>191.2 ± 46.3c</td>
<td>177.1 ± 15.8z</td>
</tr>
</tbody>
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Senile vs osmotic cataract; catalase p <0.001, GPX p <0.001, SOD p <0.001; x p >0.05, y p >0.05, z p >0.05

n = 27
SOD were observed in subjects with cataract when compared with non-cataract subjects (Table 1). In the diabetic group, no significant changes were observed in catalase, GPX, and SOD activities between cataract and non-cataract subjects. Comparisons between non-diabetic cataract (senile cataract) and diabetic cataract (osmotic cataract) groups indicated significant decreases in catalase, GPX, and SOD activities in the non-diabetic cataract group. Positive correlations were observed between erythrocyte catalase and SOD ($r = 0.75$), catalase and GPX ($r = 0.63$), and SOD and GPX ($r = 0.59$) in the subjects with senile cataracts.

**Discussion**

The present study investigated 3 antioxidant enzymes, catalase, GPX, and SOD, with respect to cataract in diabetic and non-diabetic groups of Sri Lankans. Oxidative stress has been implicated in cataractogenesis [4]. Direct estimation of blood oxidant levels is difficult because of the very short half life of free radicals; however, oxidative stress can be estimated indirectly by measuring levels of antioxidants in blood [11] or in erythrocytes [12]. The important antioxidant enzymes in the erythrocytes are catalase, GPX, and SOD [13,14].

Previous studies have reported that senile cataractous lenses are associated with decreased levels of SOD, GPX, and catalase [15-18]. A cataractous lens is likely to reflect a subject’s metabolic state, particularly the antioxidant enzyme activities in the lens at the time of development of cataract, and may therefore have less significance in the identification of risk factors. Hence, the need to investigate the blood antioxidant enzyme levels of cataract patients. A definite relationship between blood antioxidant enzyme levels and the incidence of cataracts could provide a useful marker in the identification of subjects predisposed to cataracts.

Results of the present study demonstrate significant decreases in erythrocyte catalase, GPX, and SOD activities in patients with senile cataract (non-diabetic cataract) when compared with the controls (non-diabetic non-cataract). There is significant positive correlation between erythrocyte antioxidant enzymes in subjects with senile cataracts. Our results confirm some previous findings that correlate with human cataract [19,20]. It is suggested that a decrease in the antioxidant status of the erythrocytes may increase the oxidative damage in tissues, including the oxidative modification of lens proteins observed in cataract.

However, in contrast to these data, increased blood levels of antioxidant enzymes have been reported to be associated with cataract [21-23]. This is thought to be a defensive response to increased levels of oxidation within the body. There may be a synergistic effect between the intracellular antioxidant enzymes and extracellular and membrane bound antioxidants such as ascorbate, vitamin E, and beta-carotene; low levels of these vitamins and high levels of antioxidant enzymes could minimize oxidant damage [24]. Therefore it is appropriate to consider the total antioxidant status in the interpretation of these results.

Among the diabetic subjects, no significant differences in erythrocyte catalase, GPX, and SOD activities were observed between the cataract and the non- cataract groups, indicating a non-significant role for these enzymes in the pathogenesis of diabetic cataract. Further, it was observed in this study that diabetic cataract is associated with higher levels of catalase, GPX, and SOD activities than senile cataract. These results are in agreement with a previous study, which showed that diabetic cataract is associated with higher levels of GSH (reduced glutathione) and lower levels of lipid peroxidation in the erythrocytes as opposed to senile cataract, indicating a non-significant role for GSH in the pathogenesis of diabetic cataract [Chandrasena LG. The changes in oxidation reduction status of erythrocytes in the development of cataract in diabetic and non diabetic subjects. Presented at the Annual Meeting of the American Association for Clinical Chemistry, 25-29 July 2004, Los Angeles, CA].

The influx of glucose into the diabetic lens, and its oxidation through the polyol pathway, leads to the accumulation of sorbitol in the lens, which generates an osmotic stress that may be a major contributory factor in the development of diabetic cataract [25]. Further, there is variation in the activities of antioxidant enzymes reported for diabetic cataract [25,26]. Antioxidant medications, systemic diseases, and long term complications of diabetes such as non-enzymatic glycation and autoxidation of glucose may have significant effects.
on the antioxidant status of diabetic subjects. However, chronic oxidative stress generated by the polyol pathway is likely to be an important contributory factor in the slow and progressive development of diabetic cataract.

In summary, erythrocyte antioxidant enzyme activity levels reflect the changes taking place in the development of senile cataract. Assays of these enzyme activities could provide a marker for the early detection of senile cataract.

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References