A study on role of plasma super oxide dismutase (sod) and serum Na⁺ levels in diabetic cataract patient: Comparative study

Dr. Chitteti Bhanu Kumar

DOI: https://doi.org/10.33545/pathol.2019.v2.i1f.99

Abstract
Background: Cataract is the most common cause of blindness which is treatable. Though there are multiple risk factors involved, exact pathogenesis of cataract is yet to be established. Diabetics are known to be associated with electrolyte disturbances, like hypo/hypernatremia, hyperkalemia. We hypothesize that serum electrolytes and oxidative marker enzyme super oxide dismutase (SOD) levels may be altered in diabetic cataract patients. The aim of the study was to compare serum electrolytes (Na), blood glucose and SOD in healthy controls and diabetic cataract patients as well as to assess the correlation of duration of diabetes with electrolytes as well as risk of cataractogenesis in diabetics.

Methods: SOD was measured as indicator of oxidative stress and serum sodium levels estimated in diabetic cataract patients and healthy controls.

Results: The present study involved 60 subjects, out of which 20 were Diabetic cataract cases who attended outpatient and inpatient department of Tertiary care Eye Hospital and outpatient department of RIMS & General Hospital, KADAPA, who fulfilled the inclusion criteria and other 40 were controls without diabetes and cataract. The present study was undertaken to evaluate the Blood Glucose levels, Plasma SOD levels, Serum Na⁺ levels in control and diabetic cataract patients. In the present study, Comparison of plasma SOD and blood Glucose levels between cases and controls. All the controls were having normal blood Glucose levels (non-diabetics) and normal SOD levels except for 7 controls with decreased SOD levels (12%). Among cases all are diabetic and majority 20 (50%) were with decreased SOD levels. In the present study, Mean ± SD for plasma Glucose levels for diabetic cataract cases is 217.62±55.85 and for controls is 105.86±61.75. Mean ± SD of Blood Glucose is higher in cases than in controls and the mean difference is statistically significant (p<0.01**). In the present study, the mean of serum Na⁺ in diabetic cataract cases is (147.5±6.6) and for control group is (143.4±6.0), which shows significant difference (p<0.05*). Though the mean of serum Na⁺ of the patients were slightly higher than normal range (135-145 mEq/l) but in comparison with control group, the serum Na⁺ of the patients was elevated, which notifies significant difference.

Conclusions: The present study concluded that, we observed there were significant differences in SOD levels and Na levels compared both in Diabetic cataract patients and healthy controls. The results clearly indicate that significant decrees in antioxidant enzyme in cataract patients and sodium levels increased in cases. These observational studies clearly indicate that Diabetes associated oxidative stress and electrolyte imbalance important for formation of early cataract.

Keywords: Diabetes, sodium, cataract, super oxide dismutase, antioxidant enzymes

Introduction
Diabetes mellitus is a group of metabolic disorders mainly characterized by hyperglycemia which occurs due to body’s inability to synthesize insulin or utilize insulin to its full potential [1, 2]. It is a lifelong progressive metabolic disease affecting more than 230 million people worldwide and this number is expected to reach 350 million by year 2025. It is the fourth leading cause of death by disease globally and has become one of the most challenging health problem of 21st century [2, 3]. Cataract is a leading cause of visual disability and blindness throughout the world. 1 the process of cataract formation is more pronounced and is accelerated in patients suffering from diabetes. 2 During diabetes, an increased production of reactive oxygen species (ROS) and an enhanced concentration of thriobarbituric acid-reactive substances (TBARs) resulting in oxidative stress have been observed. Cellular defense mechanism plays an important role in defense against cataractogenesis, as it protects the lens against the toxic effects of oxidative insult.
The two major anti-oxidant enzymes, which protect lens proteins against ROS mediated oxidative damage, is Superoxide dismutase (SOD). 4 There are strong evidences to show that diabetes and oxidative stress are significantly associated with each other and therefore oxidative stress plays an important role in the pathogenesis of various diabetic complications. In diabetic patients, the process responsible for cataract starts very early and worsens over the course of the disease due to overproduction of ROS and decreased efficiency of antioxidant defences which leads to increase in the level of oxidative stress.

Superoxide dismutase (SOD, EC 1.15.1.1) is an enzyme that catalyzes the dismutation of the superoxide (O\textsuperscript{2-}) radical into either ordinary molecular oxygen (O\textsubscript{2}) or hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}). Superoxide is produced as a by-product of oxygen metabolism and, if it is not regulated, can lead to cell damage. H\textsubscript{2}O\textsubscript{2} is also damaging, but less than O\textsubscript{2} and is degraded enzyme such as catalase. Thus, SOD is an important first line antioxidant defence in all living cells which are exposed to oxygen.

Cataract is a multifactorial disease, and is associated with oxidative stress and antioxidant enzymes defences against ROS, with significant decrease in the activity of superoxide dismutase (SOD), catalase, and glutathione peroxidase. And among the antioxidant enzymes, SOD is considered to be important and first line of defence against ROS. SOD protects the lens and the epithelial cells of lens from oxidative damage.

Sodium is the chief cation of extracellular fluid. Human body contains approximately 1.3gms of sodium out of which, 50% present in bones, 40% in ECF and remaining 10% in soft tissues. Normally to maintain lens membrane permeability, water electrolyte balance must be maintained intracellularly and also extracellularly. Membrane permeability is responsible for maintenance of lens transparency. Sodium is major serum extracellular cation, but in lens, concentration of sodium is less than potassium while in serum it is vice versa. This cation balance maintains osmotic pressure and thus water balance across the lens membrane with the action of Na-K\textsuperscript{+} ATPase. Rewatkar M et al. \cite{1} observed changes in serum electrolyte levels can induce changes in aqueous humour electrolytes levels of lens and result in cataract formation. Few of the studies have shown that there is significant difference in serum electrolytes concentration in cataract patients when compared to those without cataract.\cite{2} whenever there is a change in serum Na\textsuperscript{+} levels, there is a change in Na\textsuperscript{+} levels in aqueous humour as it is derived from serum. So, derangement in serum electrolytes appears to be one of the risk factor for cataractogenesis.

The aim of the present study to compare the levels of oxidative marker enzyme (SOD) levels and serum Na\textsuperscript{+} levels in diabetic cataract patients and normal healthy individuals

Materials and Methods
Study design and ethical clearance
This present observational study was done at RIMS & General Hospital, KADAPA, one year from March 2017-Feb 2018, after getting approval from Institutional ethics Committee of RIMS& General hospital KADAPA. Diabetic cataract patients attending OPD of RIMS hospital and inpatients and outpatient Tertiary care eye hospital Kadapa were taken into this study.

Blood samples of diabetic cataract patients collected for the estimation of Superoxide Dismutase (SOD), Serum Na\textsuperscript{+} levels, Blood Glucose levels, at clinical chemistry laboratory, Gandhi Hospital attached to Rajiv Gandhi Institute of Medical Sciences, Kadapa were processed.

Sample size
Total 60 patients (20 cases and 40 healthy controls) 20 cases of diabetic cataract and 40 controls (without diabetic cataract) were selected.

Selection criteria of patients
Inclusion criteria
Patients with cataract aged 35-65 years diagnosed by distant direct ophthalmoscope. Patients with known history of diabetes mellitus

Exclusion criteria
Patients aged less than 35 and more than 65 years. Patients with hypertension, past history of trauma or infection to the eye, past history of ocular surgeries and high urea and creatinine more than 1.3 were excluded.

Biochemical Parameter estimation
Plasma SOD, Serum Na\textsuperscript{+}, and Blood Glucose in the present study in 100 samples by the following methods, Superoxide Dismutase (SOD) ® Xanthine Oxidase method, Serum Na\textsuperscript{+} levels ® Ion exchange electrode method, Blood Glucose ® Glucose Oxidase-Peroxidase (GOD-POD) method.

Sample collection storage
Blood samples were collected centrifuged – serum and plasma was separated and stored in refrigerator at 2-8°C and were analyzed in batches.

Assay of Superoxide dismutase
The role of superoxide dismutase (SOD) is to accelerate dismutation of the toxic superoxide radical (O\textsubscript{2}'), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl) 3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT under the conditions of the assay. Superoxide dismutase activity was measured by the degree of inhibition of the reaction. Values are expressed as U/ml. The percent of inhibition was calculated as follows:

\[
\% \text{ Inhibition} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100 \%
\]

Statistical analysis
The results were tabulated in master chart and statistically analyzed using Microsoft Excel 2010 and department of community medicine, Gandhi Medical College Secunderabad. Results on continuous measurements are presented on Mean and SD and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance.
Results
The present study involved 100 subjects, out of which 25 were Diabetic cataract cases who attended outpatient and inpatient department of Sarojini Devi Eye Hospital and outpatient department of Gandhi Hospital who fulfilled the inclusion criteria and other 75 were controls without diabetes and cataract. The present study was undertaken to evaluate the Blood Glucose levels, Plasma SOD levels, Serum Na⁺ levels in Diabetic Cataract patients. In the present study, Plasma SOD was done by Xanthine Oxidase method, Blood Glucose by GOD-POD method and serum Na⁺ levels by Electrolyte analyzer.

Table 1: Comparison of Mean and Standard Deviation of SOD between Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=40)</td>
<td>190.44</td>
<td>19.58</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cases (n=20)</td>
<td>150.04</td>
<td>19.29</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of plasma SOD between cases and controls is shown in table 2. By dividing into 3 groups based on age group as shown in table 3 as Group I (35-45years), group 2 (46-55years), group 3 (56-65years).

Table 2: Comparison of serum SOD levels in different age groups in controls and cases

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=40)</td>
<td>105.56</td>
<td>14.72</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cases (n=20)</td>
<td>217.82</td>
<td>53.75</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of plasma SOD and blood Glucose levels between cases and controls is shown in Table 4. All the controls were having normal blood Glucose levels (non-diabetics) and normal SOD levels except for 9 controls with decreased SOD levels (12%). Among cases all are diabetic and majority 20 (80%) were with decreased SOD levels.

Table 3: Comparison of serum SOD levels in different age groups in controls and cases

<table>
<thead>
<tr>
<th>Age group (YRS)</th>
<th>Cases</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>175.3</td>
<td>190.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2</td>
<td>165.1</td>
<td>185.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3</td>
<td>142.7</td>
<td>162.7</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Group 1, Z = 4.6 p < 0.01, Significant
Group 2, Z = 7.0 p < 0.01, Significant
Group 3, Z = 2.4 p < 0.01, Significant

All the cases are with diabetes and all the controls are normal without diabetes and cataract. Mean ± SD for plasma Glucose levels for cases is 215.72±51.85 and for controls is 107.76±13.82 and the difference of mean of cases and controls is 107.96, with Z-score value being 10.3 Mean ± SD of Blood Glucose is higher than in controls and the mean difference is statistically significant (p<0.01**).

Table 4: Mean ± SD of Plasma SOD (U/ml) and Blood Glucose (mg/dl) among cases and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases Mean ± SD</th>
<th>Controls Mean ± SD</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sod</td>
<td>158.02 ± 19.29</td>
<td>185.43 ± 18.48</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glucose</td>
<td>217.62 ± 55.85</td>
<td>105.86 ± 61.75</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Discussion
An imbalance of antioxidants and pro-oxidants results in increase in oxidative stress. It is not possible to avoid secondary oxidations that are involved in ordinary metabolism since oxygen is a strong oxidant. Common ocular diseases such as age-related macular degeneration, retinopathy of prematurity and age-related cataract occurs because of involvement of oxidative stress. Diabetes seems to be associated with oxidative stress and osmotic stress in the mechanism of cataractogenesis.

It has been observed that free oxygen radicals trigger cataract which is one of the degenerative manifestations of diabetes. The high enzymatic antioxidants (SOD and catalase) levels neutralize the toxic effects of the reactive oxygen species in the lens which prevents cataract formation. The decrease in these enzyme activities in the lens and aqueous humor during ageing are responsible in the development of senile cataract.

In the present study, the statistical analysis of the obtained values showed that the Plasma SOD values are significantly lower in diabetic cataract cases (158.02 ± 19.29 units/ml) compared to controls (185.43 ± 18.48 units/ml). The mean difference was significant at p-value <0.01 (Table 5)

A comparison of serum SOD in different age groups in controls and cases was done. Both controls and cases were divided into three groups according to age distribution shown in Table 1 and compared with each other. In controls, no statistical difference was observed among different age groups when compared with one another, but in cases as the age group is increasing there is slight decrease in levels of SOD but not with high statistical significance, but there was a high statistical significance for SOD levels was observed when cases were compared with the controls of similar age (Table 3) with levels significantly lower in cases (p<0.01**).

The statistical analysis of the obtained values showed that the Plasma SOD values are significantly lower in diabetic cataract cases compared to controls and the mean difference was significant at p-value <0.01**.

In the present study, all the cases are diabetic and with cataract and all the controls are non-diabetic and without cataract. The statistical analysis of the obtained values showed that Mean ± SD for blood Glucose levels for cases is 217.62±55.85 and for controls (non-diabetic) is 105.86±61.75. Mean ± SD of Blood Glucose is higher in cases than in controls and the p-value being <0.01**

The present study revealed that, the statistical analysis of the obtained values showed that the Mean ± SD of SOD and Blood Glucose levels for cases (158.02±19.29) and (217.62±55.85) and the Mean ± SD of SOD and Blood Glucose levels for controls (185.43±18.48) and (105.86±61.75). The mean difference was significant at P value less than 0.01 that is p<0.01**.

In this study, according to above observation there is a negative correlation between blood Glucose levels and plasma SOD levels. As, the blood Glucose levels are increasing there is decrease in plasma SOD levels in cases.
but in controls it appears to be mostly within normal range as all the controls are non-diabetic.

With respect to plasma SOD in diabetics, the present study has revealed that the SOD levels were significantly low in diabetics when compared to control subjects. There is a negative correlation between two parameters. So the results are in accordance with the above studies. The formation of cataract in diabetes appears to be associated with oxidative stress. Oxidative stress is due to overload of reactive oxygen species (ROS) that leads to pathophysiology of many diseases like cataract and diabetes. It is said that free radicals will trigger cataract, one of the micro vascular complication of diabetes. The toxic effects produced by ROS are neutralized by the enzymatic and non-enzymatic antioxidants. The first order enzymatic antioxidant superoxide dismutase activity (SOD) is decreased in the lens during diabetes and is related in the development and progression of cataract. There may be two reasons for lowering of SOD in our study; 1) More and more ROS like O$_2$ are produced because of oxidative stress created by hyperglycemia, as a result SOD is being used up in the process when it converts O$_2$ to H$_2$O$_2$, and H$_2$O$_2$ also causes inhibition of SOD activity. 2) Hyperglycemia will result in glycation of enzymes and their inactivation. Glycation is because of post-translational modification in vivo, which leads to aggregation, crosslinking and insolubilization of lens proteins. Studies have shown that glycation will cause damage to lens proteins and thus affecting their normal function. In the present study, the activity of SOD are lower in diabetic patients when compared to normal subjects this is because diabetes leads to increased formation of ROS and AGEs when compared to normal individuals. This glycation not only inactivates SOD but also inactivates other enzymes like catalase which is responsible for the removal of intracellular H$_2$O$_2$. So, H$_2$O$_2$ is not detoxified can act as inhibitor of SOD. Thus, inactivation of Catalase not only contributes towards increased level of Superoxide radicals but also causes SOD inactivation. In the present study, there was a significant increase in blood glucose levels in Diabetic cataract patients; these levels are associated with some complications of diabetes. These findings similar with previous studies, et al. investigated at increased glucose levels caused inactivation of SOD and catalase and the loss of antigenicity and these lead to the loss of activity of both the enzymes.

The decreased SOD level in patients (cases) may indicate shifting of redox balance towards oxidative stress leading to development of cataract. In the same way, higher level of SOD may have prevented the development of cataract in controls. In this present study, in diabetic cataract patients, it has been found that levels of plasma SOD were low and the study supports the hypothesis that decreased antioxidant enzyme activities are associated with diabetes and cataract development.

In the present study, the statistical analysis of the obtained values showed that the Mean ± SD of serum Na$^+$ levels for cases (143.5±6.0) and for controls (141.4±6.0) and difference of means of cases and controls are 3.0 with Z − score value being 2.43. Mean ± SD of serum Na$^+$ level between the two groups is statistically significant ($p<0.05^*$). In the present study, the mean of serum Na$^+$ in diabetic cataract cases is (147.5±6.6) and for control group is (143.5±6.0), which shows significant difference ($p<0.05^*$). Though the mean of serum Na$^+$ of the patients were slightly higher than normal range (135-145 mEq/l) but in comparison with control group, the serum Na$^+$ of the patients was elevated, which notifies significant difference. These findings were similar with that of previous studies, , in his study concluded that, any alteration in serum Na$^+$ levels will affect the aqueous humour Na$^+$ levels. Hence, there is an alteration in their ratio in cataract patients. As this fluid itself derived from serum, any alteration in serum electrolytes appears to be one of the risk factor for cataractogenesis.

Any factors which disturb the equilibrium of water and electrolytes or damage the colloid system of lens fibers leads to opacification. Aqueous humour is a clear fluid which is secreted by the ciliary epithelium from the serum in the eye. Lens derives its nutrition from the aqueous humour. Since lens metabolism is associated with aqueous humor secretions, serum electrolytes concentration may directly affect electrolytes of aqueous humor fluid and in turn lens metabolism. This also gain support from the finding of New York Eye Study. In the present study, Na$^+$ levels are higher in cases when compared to controls. It is correlating with above studies. Blood Glucose may impact serum Na$^+$ levels. Some previous studies reported that the blood sugar level was higher in the cataract group than in the control group. This major finding correlated with other studies and observed that glucose as initiating factor when the lens react to glucose and protein molecules lead to production of intraocular mass which disrupt lens fibers thus damaging the inorganic ion balance. Diabetic patients develop a series of electrolyte disorders. Diabetes is a well-known cause of dysnatremia via several underlying mechanisms. The development of hypernatremia is associated with endocrine dysfunction. Glucose is an osmotically active substance. There is some evidence in man that hypernatremia and hyperosmolarity are associated with impairment of both insulin-mediated glucose metabolism and glucagon-dependent glucose release.

**Conclusion**

One of the most important findings in the current study is that decreased serum levels seem to be associated with cataract development in diabetic patients. In the current study, cataract patients with diabetes had low levels of SOD and increased electrolyte balance (Na levels) as compared to cataract patients without diabetes. However, our results show that serum levels have an association with early cataract development in diabetics. Hence a diet low in sodium could be protective against cataract, conversely a diet high in sodium may act as a risk factor for cataractogenesis. This study shows that increased oxidative stress and decreased antioxidant enzyme activities and electrolyte imbalance have a role in the development of cataractogenesis.

**Acknowledgement**

The author thankful to department of Biochemistry, Gandhi Institute of Medical Sciences, Kadapa for providing facilities to carry out this work.
Conflict of interest
The authors have no any conflict of interest.

References