RELATIONSHIP BETWEEN OXIDATIVE STRESS AND PRIMARY OPEN-ANGLE GLAUCOMA

Afreen Hasan1, Pragati Garg2, Anu Chandra3, Mohit Gupta4

1Senior Resident, Department of Biochemistry, Era Lucknow Medical College, Lucknow, Uttar Pradesh.
2Professor and HOD, Department of Ophthalmology, Era Lucknow Medical College, Lucknow, Uttar Pradesh.
3Professor, Department of Biochemistry, Era Lucknow Medical College, Lucknow, Uttar Pradesh.
4Junior Resident, Department of Ophthalmology, Era Lucknow Medical College, Lucknow, Uttar Pradesh.

ABSTRACT

BACKGROUND
Primary Open-Angle Glaucoma (POAG) is a progressive chronic optic neuropathy, which is not alone related to intraocular pressure. Oxidative stress in conjunction with vascular abnormalities, glial activity, immune system and inflammatory stimulus are presumed to be involved in glaucoma-induced injury.

The aim of the study is to evaluate the role of oxidative stress in POAG.

MATERIALS AND METHODS
It is an observational study conducted on 60 individuals of more than 40 years of age after ethical clearance and informed consent. 30 patients were confirmed POAG and 30 were age-matched controls of either sex. Patients having angle closure glaucoma, secondary glaucoma or congenital glaucoma, evidence of hepatic and renal diseases, laser and ocular surgeries, any degenerative disorder, age-related macular degenerations or ocular disease, which may increase Intraocular Pressure (IOP) were excluded. 5 mL blood of all patients was taken under aseptic condition in EDTA tube and sample was transferred to biochemistry lab. Parameters assessed were Thiobarbituric Acid-Reactive Substance (TBARS), global antioxidant activity by FRAP method, antioxidant enzymes- superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), ancillary antioxidant enzyme Glutathione Reductase (GR) and Reduced Glutathione (GSH) in hemolysate.

RESULTS
No significant difference was seen between case and controls with regard to SOD, GSH or CAT levels but GPx activity was raised in POAG in case as compared to controls. Also, very low Total Antioxidant Activity (TAA) in both case and control are noticed.

CONCLUSION
The study indicates that oxidative stress was not a risk factor in patient at the point of stage at which they were examined. Thus, it is required that further longitudinal study should be undertaken to examine oxidative stress.

KEYWORDS
Oxidative Stress, Primary Open-Angle Glaucoma.


BACKGROUND
POAG is defined as a chronic progressive optic neuropathy in adults where Intraocular Pressure (IOP) and other currently unknown factors contribute to damage and in which in absence of other identifiable causes, there is characteristic acquired atrophy of optic nerve and loss of retinal ganglion cell and their axons.

The prevalence of POAG varies in different population.

In general, it affects about 1 in 200 of population (of either sex) above the age of 40 years. Prevalence of POAG is 3-4 times higher in blacks than in Caucasians.1

The exact sequence in which the disease starts is not known, but it is a fact that glaucoma is not a condition relating to IOP alone. Oxidative stress in conjunction with vascular abnormalities, glial activity, immune system and inflammatory stimulus are all presumed to be involved in glaucoma-induced injury. Therefore, the “trait de union” of all these components is called the oxidative stress.2 Oxidative DNA damage is an inevitable consequence of cellular metabolism and it is secondary to free radical formation.3 The major types of free radicals and their non-radical reactive species are Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). ROS and RNS are reported to be culprits (risk factors) in POAG. To cope with the ROS, human cells express an array of antioxidant enzymes such as Mn2+ dependent Superoxide Dismutase (SOD), copper/zinc SODs, glutathione peroxidase, glutathione reductase and Catalases (CAT). Many of the ROS
mediated responses actually protect the cells against oxidative stress and establish ‘redox homeostasis’.4

Interestingly, in ageing process, there is an age dependent increase in ROS and RNS production that may escape cellular defence mechanism and exert damage to cellular constituents, including DNA, RNA, lipid and proteins.

Therefore, a signal or stimulus that triggers over production of ROS or RNS is likely to induce the optic nerve injury.

Total antioxidant power and endogenous antioxidant can be taken as determinant for POAG and as these are the factors, which are present genetically in RBCs and plasma, it can be reflected in blood and proved to be an early indicator of POAG.

With this background, we conducted this study with an aim to evaluate the role of oxidative stress by measuring Thiobarbituric Acid Reactive Substance (TBARS) in terms of Lipase Peroxidase (LPO) and antioxidant enzymes namely catalase, SOD, GPX and GR in POAG patients and healthy control group above the age of 40 yrs. of either sex.

**MATERIALS AND METHODS**

This observational study was conducted in a tertiary hospital in a time duration of 18 months. 60 individuals above 40 yrs. of age, irrespective of sex were included after ethical clearance and informed consent of the person. They were equally divided into two groups, one with POAG and other without POAG. Inclusion criteria for POAG was IOP >21 mmHg with possible glaucomatous visual field defects, Cup-Disc Ratio (CDR) higher than 0.5 or difference of CDR of 0.2 between two eyes. Patients that were excluded were those having acute angle closure glaucoma, secondary glaucoma or congenital glaucoma; evidence of renal or hepatic disease; having acute angle closure glaucoma; renal or hepatic disease; acute angle closure glaucoma; or any other ocular condition.

In all these patients, detailed history was taken and complete ophthalmological examination was done to diagnose POAG and to rule out all other conditions, which could hamper the biochemical values of oxidative enzymes.

5 ml of venous blood of all the patients was taken under aseptic conditions in EDTA vials. The blood was centrifuged, plasma separated and used for estimation of TBARS in terms of Malondialdehyde (MDA) and Total Antioxidant Activity (TAA) in terms of Ferric Reducing Antioxidant Power (FRAP) assay.

After this, RBC lysates were prepared for estimation of antioxidant enzymes and compound, namely Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX), Glutathione Reductase (GR) and Reduced Glutathione (GSH).

The data was compiled and analysed using SPSS software version 17/MS Excel student unpaired t-test was done and P value of <0.05 was considered as statistically significant.

**RESULTS**

In our study, we have assayed the various enzymes in 60 patients, in which 30 were POAG patients (Group I) and 30 were age-matched controls without POAG (Group II) of either sex. The parameters, which were analysed were-Thiobarbituric Acid-Reactive Substances (TBARS), Global antioxidant activity by FRAP method, antioxidant enzymes-Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and Catalase (CAT) (all in hemolysate) and ancillary antioxidant enzyme like Glutathione Reductase (GR) and Reduced Glutathione (GSH) in hemolysate.

Age of patients ranged from 40-80 years with mean age 57.1 ± 11.33 (Group I) and 57.3 ± 7.85 (Group II).

The mean level of TBARS in Group I was 4.4 ± 0.824 nmol/mL and in Group II was 4.2 ± 0.794 nmol/mL with no significant difference between the two.

The mean FRAP level was very low, i.e. below 600 µmol/L in both the groups being 1574 ± 160 µmol/L in group I and 577 ± 118 µmol/L in group II and the statistical difference in the global antioxidant activity between the two groups was found to be statistically insignificant (p = 0.922). Also, the difference in the level of SOD and in the levels of catalase enzyme among the cases and controls were insignificant (p = 0.482 and 0.600, respectively).

There was no significant difference found in the level of GSH between the two groups. Levels being 30.26 ± 3.91 mg/gm protein and 29.72 ± 3.06 mg/gm protein in group I and group II, respectively. Glutathione peroxidase (µgm GSH utilised per minute per mg of protein) value in group II was 30.88 ± 2.4 µgm, and in group I, it was found to be 33.19 ± 3.8, significant differences were seen in Glutathione Peroxidase (GPx) values and Glutathione Reductase (GR) values in group I and II having ‘p’ value 0.007 and 0.013, respectively.

While the level of Glutathione Reductase (GR) was 27.8 ± 3.72 nmol of NADPH oxidised per min. per mg protein in group II and 30.08 ± 3.17 nmol of NADPH oxidised per min. per mg of protein in group 1 (Table 2).

In group I, only a significant difference in the values of global antioxidant activity by FRAP method was found between vegetarians (n=05) and non-vegetarians (n=25). The values being 744.8 ± 182.91 µmol/L in vegetarian and 540 ± 134.61 µmol/L in non-vegetarians (p=0.007) (Table 3).

On evaluating the effect of smoking on different parameters in both the groups, no significant difference was found in enzyme levels between smokers and non-smokers. (Table 4).
DISCUSSION

POAG is an age-related neurodegenerative disease triggered by mechanical stress due to IOP, reduced blood to retina, reperfusion injury, glutamate excitotoxicity, aberrant immune response and raised oxidative stress.\(^5\) Multiple risk factors can cause retinal ganglion cell loss including apoptosis. It has been proposed that oxidative processes may trigger and mediate apoptotic death of RGC during reoxygenation.\(^6\)

Also, it has been postulated, but not proven that dietary antioxidants or antioxidant supplemetations may enhance trabecular meshwork function and protect the optic nerve;\(^7\) but most of these assumptions are just guesses with no definite conclusions.

In our study, we have included the diagnosed case of POAG having a mean age of 57.1 yrs. Nearly, 43% patients were above 60 years, 20% were between 50-60 years and 36.67% were between 40-50 years.

This observation strongly favours that POAG is an age-related disease. No correlation was found between age and oxidative stress in our study, which indicates that other risk factors are also involved and must be contributing in etiopathogenesis of POAG.

Neurodegenerative disease usually have raised OS, which may begin with the onset of the disease or may develop on some later date.\(^8\) OS has been measured by us as Thiobarbituric Acid Reactive Substance (TBARS), which are representative of Malondialdehyde (MDA). MDA is an end product of polyunsaturated fatty acid especially arachidonic acid in human body.\(^9\)

In our observation, we have not found raised OS in POAG patients, although overall data suggests the possibility of raised oxidant activity.

Although, numerous studies have suggested that raised OS is involved in etiopathogenesis of POAG, but no explanation is available in literature about the mechanism.

Although, the mean level of TBARS in POAG patients was nonsignificant, but it was very slightly higher (4.4 ± 0.824 nmol/mL) than controls (4.2 ± 0.794 nmol/mL). While comparing the two groups the difference was insignificant when 5.0 nmol/mL was taken as index. In Group I, 23% (n=7) patients had level above 5.0 nmol/mL and 77% (n=23) had level below it. 77% patients showed mild stress.

**Table 1. Mean Age and IOP in Study Group**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Case (Mean ± SD)</th>
<th>Controls (Mean ± SD)</th>
<th>t-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO (nmol MDA/mL plasma)</td>
<td>4.439 ± 0.824</td>
<td>4.222 ± 0.794</td>
<td>-1.038</td>
<td>0.304</td>
</tr>
<tr>
<td>SOD (unit/mg protein)</td>
<td>1.257 ± 0.227</td>
<td>1.306 ± 0.304</td>
<td>0.707</td>
<td>0.482</td>
</tr>
<tr>
<td>CAT (unit/mg protein)</td>
<td>51.356 ± 6.050</td>
<td>50.655 ± 4.039</td>
<td>-0.527</td>
<td>0.600</td>
</tr>
<tr>
<td>GPx (umg GSH utilised/min./mg protein)</td>
<td>33.193 ± 3.832</td>
<td>30.876 ± 2.399</td>
<td>-2.806</td>
<td>0.007</td>
</tr>
<tr>
<td>GR (nmol of NADPH oxidised/min./mg protein)</td>
<td>30.075 ± 30.166</td>
<td>27.799 ± 3.716</td>
<td>-2.554</td>
<td>0.013</td>
</tr>
<tr>
<td>GSH (mg/gm protein)</td>
<td>30.264 ± 3.913</td>
<td>29.721 ± 3.062</td>
<td>-0.599</td>
<td>0.552</td>
</tr>
</tbody>
</table>

**Table 2. Unpaired t-Test (Case Vs. Control)**

**Table 3. Impact of Diet on Various Parameters**

**Table 4. Impact of Smoking**

and 23% showed moderate degree of stress. While in group II, one subject showed mild degree of stress and another one showed moderate degree of stress. So, we may state that even among normal population, there are some “high generators of ROS” and require medical attention.10

We also measured TAA (total antioxidant activity) as it is a better index of global total exogenous antioxidant activity. The method used for this was “ferric reducing antioxidant power” procedure by Benzie and Strain (1996).11 The normal range as reported in plasma is 612-6134 μmol/L. It measures dietary antioxidant in plasma along with some endogenous antioxidants of lesser concentration (uric acid and bilirubin, etc.) when total nonenzymatic capacity is counted. The method suffers from two defects- first, it does not measure enzyme antioxidants, and second, it also does not measure most important thiol compounds including GSH, which acts both as cofactors of antioxidant enzymes and also acts independently as antioxidants. In both groups, FRAP level was even below 600 umol/L (group I = 574 ± 160 umol/L; group II = 577 ± 118 umol/L).

These findings point out that majority of both groups suffered from decreased non-enzymatic antioxidant activity where nutrient antioxidant vitamin C and E are major contributors. Another important observation we came across was that vegetarian Group I patients had significantly higher FRAP values (744 ± 182 micromol/L) than non-vegetarian patients (540 ± 134 micromol/L). The values of vegetarian and non-vegetarian patients in controls did not differ in this regard. It maybe because vegetarians were taking more of fruits and green vegetables, which are rich in dietary antioxidants.

Both raised and low value of total antioxidants are reported in literature in POAG patients. TAS decreased in the plasma of POAG patient and that maybe a risk factor in the pathogenesis of POAG.12 Sorkhabi et al, 2011, have presented supporting data. Their findings are significant to point out that oxidative DNA damage increases and TAS decreases in the serum and aqueous humour of glaucoma patients. They suggested that their finding may have an important bearing on the pathogenesis of glaucoma. Tanito et al from Japan also reported lower serum total antioxidant activity in POAG patients, thus supporting above claims.

In our study, we found no difference in the level of SOD among group I (1.26 ± 0.23 units) and group II (1.31 ± 0.30 units). No effect of diet on SOD was also observed. However, more importantly, no difference was observed between smokers and nonsmokers in both groups.

In group I, the activity of GPx and GR was significantly raised. This observation with regard to GPx and GR conveys very important information. They clearly point out “raised antioxidant activity.” Significantly, raised values of GPx was observed in hemolysate group I patients. It can be safely assumed that normal level of SOD was able to scavenge this slightly enhanced level of H2O2, hence the activity of SOD was not raised.

Reduced Glutathione (GSH) is the most powerful thiol antioxidant with enormously wide range of biological activities. About 50% of the requirement is provided by diet and remaining 50% is synthesised in vivo. Fruits and vegetables enhance GSH status. In my study, the GSH level in RBC’s was 29.7 ± 3.1 mg/100 mL and 30.3 ± 3.9 mg/100 mL in group II and group I respectively and showed no difference. Further, no effect of diet or smoking was visible. Gherghel et al13 noted that POAG patients suffered from significantly lower level of blood GSH levels and redox index.

**CONCLUSION**

We found that there is mildly raised oxidant activity in POAG patients. It was significantly indicated by raised glutathione peroxide and glutathione reductase activities. However, the normal SOD activity (no difference between POAG patients and controls) was noted. The average total antioxidants capacity as measured by FRAP method was very low in both the groups.

As considerably low levels of reduced glutathione was present in both the groups and as 50% of GSH is provided by diet, the finding suggest glutathione rich diet or pure glutathione capsules should be supplemented.

In view of the results obtained in our study, it required that the longitudinal studies should be undertaken to assess oxidative stress at different time intervals and also to evaluate the effect of dietary supplements on TPA and GSH values.

**REFERENCES**


