

**IJCRR**

Vol 04 issue 24

Section: Healthcare

Category: Research

Received on: 06/10/12

Revised on: 02/11/12

Accepted on: 25/11/12

HOMOCYSTEINE LEVELS AND ROLE OF OXIDATIVE STRESS IN PRIMARY OPEN ANGLE GLAUCOMA

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ABSTRACT

Objective: Primary open angle glaucoma is the most commonest form of glaucoma which leads to vision loss if untreated. It has been hypothesized that oxidative damage may be involved in the pathogenesis of glaucoma and homocysteine may also have a role in development of glaucoma. The aim of our present study is to evaluate the role of oxidative stress and Homocysteine in Primary open angle glaucoma patients.

Methods: We tried to assess Serum Homocysteine levels and the role of oxidative stress in patients by estimating the levels of lipid peroxidation assessing plasma Malondialdehyde (MDA) levels and antioxidant status by reduced glutathione (GSH), Vitamin-E, Vitamin-C in blood. For this, we have taken 50 cases of primary open angle glaucoma patients compared with 75 age matched controls. **Results:** There were significant increase in the levels of MDA and significant decreases in the levels of antioxidants like GSH whereas there are significant increases in the levels of vitamin-E and Vitamin-C in breast cancer patients when compared with controls. No statistical significant difference was observed with Serum Homocysteine levels in cases when compared with controls. **Conclusion:** Our results indicate that oxidative stress is associated with the development of Primary open angle glaucoma which needs further studies.

Keywords: Oxidative stress, Malondialdehyde, Reduced glutathione (GSH), Vitamin-C, Vitamin-E and Homocysteine

INTRODUCTION

Glaucoma is an eye disease in which the optic nerve is damaged in a characteristic pattern leading to permanently damaged vision in the affected eyes and blindness if left untreated. Glaucoma is the second leading cause of vision loss in the world affecting approximately 70 million people worldwide.(1). Primary open angle glaucoma affects 1 to 2 percent of the population over the age of 40(2) which is the most common form of glaucoma.

Elevated intraocular pressure due to reduction in aqueous outflow facility is the most significant risk factor. High intra ocular pressure usually occurs as a result of an increase in aqueous humor outflow resistance in trabecular meshwork. Recent evidences indicate that vascular risk factors may also play a role. Impaired microcirculation and abnormal perfusion may cause glaucomatous damage in the optic nerve head. Anatomical or functional abnormalities of the vessels of the optic nerve head such as arteriosclerosis

or vascular dysregulation might be the causative factor(3,4).

Homocysteine is an intermediary amino acid formed during the conversion of methionine to cysteine. Elevated total plasma level of amino acid Homocysteine has been identified as an independent risk factor of arteriosclerosis involving coronary, cerebral, and peripheral arteries. High levels of Homocysteine causes lipid peroxidation, vascular endothelial injury, impaired vasomotor regulation, pro thrombotic surface, and therefore atherothrombogenesis.(5)

Hyperhomocysteinemia is suggested to be an independent risk factor for premature vascular disease(6), myocardial infarction(7), and stroke(8). Furthermore, raised Homocysteine levels have recently been suggested as a risk factor for non-arteritic anterior ischemic optic neuropathy(9) and retinal vascular occlusive disease with thromboembolism.(10). Recently, higher levels of plasma Homocysteine in primary open angle glaucoma(11), pseudo exfoliation syndrome and glaucoma patients(12,13) were reported.

The trabecular meshwork of patients with primary open angle glaucoma is characterized by specific morphologic and biochemical changes such as loss of trabecular meshwork cells, accumulation of extra cellular matrix, and accelerated senescence.(14). It is assumed that these changes lead to an increased outflow resistance and thus to elevated intraocular pressure. The reasons for these changes are not clear. Various factors may play an elementary role in the pathologic course of the disease, such as genetic factors, increased levels of glutamate, changes in nitric oxide metabolism, and vascular changes. One factor which is increasingly important in the pathogenesis of primary open angle glaucoma is oxidative stress.(15). Oxidative stress represents a harmful state defined by the presence of pathologic levels of reactive

oxygen species relative to antioxidant defense. Reactive oxygen species are molecules that oxidize more strongly than oxygen (O₂) itself, or molecules containing oxygen that generate free radicals. ROS include superoxide, hydrogen peroxide and hydroxyl radical, which is the strongest oxidant produced in biological systems. Other reactive species, reactive nitrogen species include nitric oxide and peroxynitrite, a product resulting from the reaction between ROS and nitric oxide.

Free radicals can be generated in an exaggerated manner and can injure tissues and organs by interacting with lipids, proteins or DNA(16). To survive, the human body has developed a complex, efficient and highly adaptive antioxidant defense system that includes two categories of antioxidants enzymatic such as glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase and non enzymatic such as reduced glutathione, antioxidant vitamins and low molecular weight compounds such as urate. The eye is also protected against oxidative stress by several mechanisms involving antioxidant enzymes such as GSH and ascorbate.(17). Altered GSH and GSH activity has been reported in the trabecular meshwork and aqueous humor of patients with glaucoma.(18).

MATERIALS AND METHODS

The present study was conducted in department of ophthalmology and the department of Biochemistry, S.V. Medical college, Tirupati. A total of 50 cases of primary open angle glaucoma patients belonging to the age group of 30-50 years were selected for this study. Age and sex matched 75 normal patients without glaucoma were selected as controls. Informed consent from all cases and controls were obtained. Ethical clearance was obtained for this study.

A detailed medical history was obtained to identify those with known or suspected diabetes mellitus, systemic hypertension, peripheral or coronary artery disease, venous thrombotic events, or cerebrovascular disease, and current drug therapy. Exclusion criteria included diabetes mellitus, systemic hypertension, peripheral or coronary artery disease, cerebrovascular disease, major systemic illness, evidence of vasculitis, renal or hepatic disease, gastrointestinal malabsorption, cardiomyopathy, psychiatric illness, chronic alcohol abuse, smoking, anticonvulsant and immunosuppressive therapy, postmenopausal hormone replacement, current use of cholesterol lowering drugs, antidepressants, antimicrobial therapy and vitamin supplements. All subjects underwent a complete ophthalmic examination including visual acuity, slit lamp examination, intraocular pressure measurement using Goldmannn applanation tonometry, gonioscopy, and dilated fundoscopic examination and systemic examination. Inclusion criteria were as follows: Primary open angle glaucoma was defined by the presence of an open angle on gonioscopy, IOP \geq 22mm of Hg measured with a Goldman Applanation Tonometer, typical glaucomatous cupping and visual field defect

in at least one eye on standard automated perimetry. Control subjects had no history of elevated IOP higher than 22 mm of Hg, no exfoliative material on the anterior lens capsule, normal visual fields and optic discs. 10ml of fasting blood samples were collected by venipuncture and for the separation of sera, 5ml of blood was centrifuged at 3000rpm for 5min and the remaining 5ml of blood was taken into a plain vial containing EDTA and was centrifuged at 3000rpm for 10min for the separation of plasma. The plasma MDA levels were estimated by using thiobarbituric acid reacting substances(TBARS) by the method of Yagi(19) and Sinnhuber et al(20). Reduced glutathione was determined by the method of Beutler et al(21). Serum Vit-E was measured by the method of Baker on the basis of reduction of ferric ions to ferrous ions by Vit-E & the formation of red colored complex with 2,2'-dipyridyl at 520nm(22,23).The activity of Ascorbic acid was determined by the method of Tietz(24). Serum total homocysteine levels were determined by enzyme linked immunosorbent assay(ELISA) kit .All the results were expressed as mean \pm SD and statistical comparisons were done using student t-test using the SPSS package.

RESULTS

TABLE 1: Comparison of levels of MDA, enzymatic and non-enzymatic antioxidants in cases and controls

Parameters	Cases(n=50)	Controls(n=75)	P value
Homocysteine (ng /ml)	8.13 \pm 2.62	7.96 \pm 2.46	Not significant
MDA (nmol /ml)	8.99 \pm 0.13	2.18 \pm 0.12	<0.001 (highly significant)
GSH (mg/g Hb)	7.37 \pm 0.21	14.66 \pm 0.13	<0.001 (highly significant)
Vitamin-C (mg/dl)	6.58 \pm 0.16	2.14 \pm 0.19	<0.05 (significant)
Vitamin-E (mg/dl)	7.63 \pm 0.11	1.32 \pm 0.28	<0.001 (highly significant)

Evaluation of oxidative stress is done based on the levels of MDA and statistically significant increase in the level of MDA was observed in open angle glaucoma patients when compared to controls. Statistically significant decreases were observed in the levels of enzymatic antioxidants like GSH and statistically significant increases in the levels of non-enzymatic antioxidants vitamin-E,C in cases when compared to controls. There was no statistical significant difference in the levels of serum homocysteine in cases when compared with controls.

DISCUSSION

Glaucoma, being a non systemic disease, is the most frequent etiology of irreversible blindness worldwide is an ocular pathology. Studies related to its effects on serum oxidative stress markers are quite limited in the existing literature. In this study, the role of homocysteine and the effects of oxidative stress and antioxidant status in glaucoma were studied. Glaucoma is an optic neuropathy, and oxidative stress plays an important role in its etiopathogenesis. In our study, a statistically significant relationship was found between the presence of primary open angle glaucoma and plasma MDA levels. The plasma MDA level, a byproduct of lipid peroxidation, is a reliable and commonly used biomarker of overall lipid peroxidation. As there are high aqueous concentrations of hydrogen peroxide and photochemical reactions in the anterior segment arising from aerobic metabolisms, the trabecular meshwork is exposed to high levels of oxidative stress.(25) Aqueous humor is known to contain several active oxidative agents such as hydrogen peroxide and superoxide anion(26).It has been suggested that chronic oxidative stress induced by such agents can compromise trabecular meshwork functions (27) and subsequently play a role

in the pathogenesis of primary open angle glaucoma. Our finding of increased plasma MDA levels in primary open angle glaucoma patients is not only consistent with the role of oxidative stress in glaucoma but also supports the idea that plasma MDA levels may be used as a marker of oxidative stress on a group basis.

We also observed significant decrease in the levels of reduced glutathione in cases when compared to controls. Circulating GSH can be depleted either by subjecting cells to oxidative stress, or by inhibition of synthesis. In patients with glaucoma, due to a high level of oxidative stress,(28) GSH could be overused implying a reduction of protection against ROS, possibly due to a defective redox cycle. Another possible explanation for reduced GSH levels may be due to defective intracellular synthesis. The liver is the major site for GSH synthesis. The precursors necessary for this synthesis are L-glutamate, L-cysteine and L-glycine. Although both glutamate and glycine are important, it seems that the major determinant of the rate of GSH synthesis is the availability of the amino acid cysteine.(29).Cysteine results from the metabolism of homocysteine and any interruption in the homocysteine-cysteine pathway would result not only in the accumulation of homocysteine but also in less available quantities from the second amino acid which may affect GSH synthesis. There is a significant increase in the levels of vitamin-E in cases when compared to controls. Vitamin-E acts as antioxidant and many in vivo and in vitro studies performed in normal and neoplastic cells have demonstrated that α -tocopherol had special effects, including gene regulation(30).This phenomenon also applies to ocular tissues.(31). It also has been established that vitamin-E derivatives act as neurohormones, and initiate various intracellular conduction pathways with a lock and key model. Retinal

blood flow regulating and neuroprotective effects of vitamin-E in glaucoma patients have been clinically demonstrated.(32). Moreover, vitamin-E is recognized for prolonging life span in retinal cell cultures(33).Taking all the above factors into consideration, the elevation of vitamin-E in glaucoma indicates the exaggerated response of antioxidants to oxidative stress in glaucoma. There is also a increase in the levels of non-enzymatic anti-oxidants such as Vit-C, which states that there is an increased defense mechanism against oxidative damage in breast cancer. The increase in the levels of these non-enzymatic antioxidant parameters may be due to an increased turnover for preventing oxidative damage in these patients, thus suggesting an increased defense against oxidative damage. Our results support the researchers who showed over expression of antioxidants(34).Several other researchers reported decreases in the antioxidant level and increases in lipid peroxidation level(35).

No statistical difference was observed in the levels of homocysteine levels in cases when compared with controls. Some studies have reported elevated serum homocysteine level in primary open angle glaucoma patients and suggested that thermo labile methylene tetrahydrofolate reductase deficiency may be in part the cause of the increased serum homocysteine level in patients with primary open angle glaucoma.(36).Our findings supports the study of researchers who observed no difference in homocysteine levels.(37).

In conclusion, the present study revealed an increase in the levels of MDA, vitamin-E,C and decrease in the levels of reduced glutathione with no alterations in the levels of serum homocysteine in cases compared to controls suggesting the role of oxidative stress as a pathogenic mechanism in the development of glaucoma and further

extensive studies are required in future to establish oxidative stress as a biomarker in the development of primary open angle glaucoma.

ACKNOWLEDGEMENTS

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors/editors/publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

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