Oxidative stress induced by posterior segment biomicroscopy; biochemical estimation of enzymatic antioxidants in serum.

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Citation

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Abstract

The present study was conducted on 36 eyes of 36 patients who attended the “Retina Centre” of Institute of Ophthalmology, Aligarh Muslim University. Informed consent was obtained from all the participants of the study and the protocol was approved by the ethical committee of the centre. Fundus Slit lamp biomicroscopy was done either with Goldmann 3 mirror contact lens or Mainster Panfundoscopic lens for a period of 20 minutes by standard illumination of slit lamp. Estimation of enzyme levels of superoxide dismutase, catalase and glutathione peroxidase was done in serum prior to exposure, 24 hours after exposure and 6 weeks after exposure.We have estimated the change in the levels of enzymatic antioxidants produced following the slit lamp light exposure of retina. The purpose was to observe the response to acute oxidative stress produced following the slit lamp light exposure of retina by the estimation of serum enzymatic antioxidants. There is increase in the serum levels of catalase, superoxide dismutase and glutathione peroxidase after 24 hours of exposure however, this increase is statistically significant only for superoxide dismutase. 6 weeks following exposure the serum levels catalase, superoxide dismutase and glutathione peroxidase are higher than the baseline value before exposure however the increase is statistically insignificant. This increase in the mean antioxidant enzyme levels could be due to tissue response to increase in the reactive oxygen species. There is no study in literature to have studied the oxidative changes produced by the exposure of retina to slit lamp light for prolonged periods.

INTRODUCTION

Oxidative stress is the disturbance in the state of equilibrium of pro-oxidant and antioxidant systems in the intact cells. Free radicals of oxygen, carbon, nitrogen and sulphur constitute the variety of reactive molecules that cause an oxidative stress to the cells. These active molecules interact with cell membranes leading to lipid peroxidation (Comporti,1985; Kappus,1985). Free radicals react with proteins producing profound changes in the structure of biological membranes, which leads to severe damage to receptors and membrane bound enzymes. It also induces enzymatic activity or changes in the conformation of proteins(Stres,1986). Nucleic acids are also a target for free radical attacks generating nucleic acid strand breaks or base modifications leading to point modifications (Stres,1986). Free radical induced lipid peroxidation, alteration in nucleic acid and protein activity and structure is responsible for cell death during oxidative stress(Harris,1992).

The free radicals may spontaneously decay but there are several defence systems that contribute to the termination of free radical reactions. They include endogenous and exogenous non –enzymatic antioxidant mediated protection and enzymatic antioxidant mediated protection. In physiological conditions these defense mechanisms maintain a low steady state concentration of free radicals in the cell and their activities are precissely regulated (Harris,1992).

Retinal photic injury from operating microscope was first reported in 1983 (Berler et.a.). Since that time, incidents of retinal photic injuries from operating microscopes continue to be reported sporadically(McDonald et.al.1983; Boldreyet.al.1984;Delaeyet.al.1984; Hupp1987; Khwarg et.al.1987; LindQuist et.al. 1986; Byrnes 1992; Davidson et.al. 1993; Gomolin et. al. 1993; Byrnes et.al. 1995). While the majority of injuries produce minimal symptoms, scotoma and permanent central vision loss have ocurred in some patients (Byrnes et.al. 1992). As is true with photochemical

damage, clinical abnormalities are not evident immediately. Retinal edema or mild pigmentary motelling becomes more apparent after one to three weeks. We have estimated the change in the levels of enzymatic antioxidants produced following slit lamp light exposure of the retina. The purpose was to observe the response of posterior segment slit lamp biomicroscopy by the estimation of serum enzymatic antioxidants

MATERIALS AND METHODS

The present study was conducted on 36 eyes of 36 patients who attended the "Retina Centre" of Institute of Ophthalmology, Aligarh Muslim University. Informed consent was obtained from all the participants of the study and the protocol was approved by the ethical committee of the centre. Fundus Slit lamp biomicroscopy was done either with Goldmann 3 mirror contact lens or Mainster Panfundoscopic lens for a period of 20 minutes by standard illumination of slit lamp. Estimation of enzyme levels of superoxide dismutase, catalase and glutathione peroxidase was done in serum prior to exposure, 24 hours after exposure and 6 weeks after exposure.

ENZYME ESTIMATION

Estimation of Glutathione Peroxide (GPx)was done by technique adapted by Paglia and Valentine[17]and modified by Bilgihan et al. Estimation of Catalase (Cat) was done by technique adapted by Aebi.[18] Estimation of Superoxide Dismutase (SOD) was done by technique adapted by Oberly and Spitz.[19]

STATISTICAL ANALYSIS

All statistics were analyzed by using SPSS for windows 11 software.

The data obtained was analysed using students "t" test. Correlations between the variables were estimated by Pearson's correlation coefficients.

RESULTS

In our study the age of the patients ranged from 18 years to 70 years. Mean age of cases was 44.6±11.7 years .Out of 36 patients there were 23 (63.9%) males and 13(36.1%) females.

The serum levels of antioxidants pre-exposure, 24 hours after exposure and 6 weeks after exposure are given in Table1. The change in serum catalase level and its statistical significance is given in Table2. The change in serum Superoxide Dismutase level and its statistical significance is given in Table3. The change in serum Glutathione Peroxidase level and its statistical significance is given in Table4.

Figure 1

Table 1

Time of Estimation	Cat *	SOD**	GPx***
Pre Exposure	3.73±1.14	3.93±1.20	4.22±1.46
24 Hours Post Exposure	3.79±1.09	4.06±1.17	4.29±1.39
6 Weeks Post Exposure	3.78±1.15	3.99±1.26	4.27±1.48

* µmol H_2O_2/min mg serum protein.
** unit/mg serum protein.
*** nmol oxidized NADPH/min/mg serum protein.

Figure 2

Table 2

Time of Estimation	Catalase	Statistical Analysis
24 Hours Post Exposure	0.06±0.23	t = 1.407 p=0.169
6 Weeks Post Exposure	0.05±0.28	t = 0.958 p=0.346

µmol H_2O_2/min mg serum protein.

Figure 3

Table 3

Time of Estimation	Superoxide Dismutase	Statistical Analysis
24 Hours Post Exposure	0.13±0.28	t = 0.05 p=0.213
6 Weeks Post Exposure	0.06±0.28	t = o.910 p=0.218

** unit/mg serum protein.

Figure 4

Table 4

Time of Estimation	Glutathione Peroxidase	Statistical Analysis
24 Hours Post Exposure	0.07±0.25	t = 1.522 p=0.138
6 Weeks Post Exposure	0.05±0.24	t =1.104 p=0.210

** unit/mg serum protein.

DISCUSSION

We have estimated the change in the levels of enzymatic antioxidants produced following the slit lamp light exposure of retina. The purpose was to observe the response to acute oxidative stress produced following the slit lamp light exposure of retina by the estimation of serum enzymatic antioxidants. Retinal photocoagulation is known to cause a temporary increase in free radical activity resulting directly in an increase in lipid peroxidation however there is no study in literature to have studied the oxidative changes produced by the exposure of retina to slit lamp light for prolonged

periods. However we have observed that the acute oxidative stress induces protective mechanism in the form of increased redox level which produces more prolonged changes in the level of antioxidant enzymes. There is increase in the serum levels of catalase, superoxide dismutase and glutathione peroxidase after 24 hours of exposure however, this increase is statistically significant only for superoxide dismutase. 6 weeks following exposure the serum levels catalase, superoxide dismutase and glutathione peroxidase are higher than the baseline value before exposure however the increase is statistically insignificant. This increase in the mean antioxidant enzyme levels could be due to tissue response to increase in the reactive oxygen species. The change induced in the redox status of the tissue may initiate intracellular signal transduction process that could trigger expression of different proteins including the antioxidants.

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