

# The effect of metal deposition on antioxidant enzymes of lens in smokers of Karachi, Pakistan

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## Abstract

**Background:** Blindness due to cataract is a major and important problem in Pakistan. The problem is expected to increase in future due to increase in tobacco smoking. The aim of study was to look for the mechanism associated with metal deposition and its effect on antioxidant enzymes in lens of smokers in Karachi, Pakistan.

**Methods:** 20 cataract patients were randomly selected from Out Patient Department (OPD) of Layton Rahmatulla Benevolent Trust Hospital LRBT Hospital, Karachi in accordance to inclusion and exclusion criteria. 10 were chronic smokers and 10 were non-smokers. After extra capsular cataract extraction, lenses were cut into two equal halves. In one half, concentration of lead, cadmium and copper were determined by Atomic Absorption Spectrophotometry; in the other half, Glutathione Peroxidase and Superoxide Dismutase were estimated by kit method using Plate Reader.

**Results:** The activity of Superoxide Dismutase and Glutathione Peroxidase were significantly decreased in lenses of cigarette smokers as compared to non-smokers. Concentration of copper, lead and cadmium were significantly higher in lenses of smokers than those of non-smokers. A highly negative correlation of both enzyme concentrations was found with concentration of copper, lead and cadmium.

**Conclusion:** Decreased level of anti-oxidant enzymes, superoxide dismutase and glutathione peroxidase with increased level of metals in smoker's lenses suggest that metals produce an oxidative stress affecting the enzyme level and its activity. These changes lead to lenticular protein changes leading to cataract.

**Keywords:** Antioxidant; Blindness; Cataract; Enzyme; Smoking

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## INTRODUCTION

Tobacco epidemic is one of the biggest threats the world has ever faced which mainly affects the third world countries (1). It is predicted that by the year 2020, tobacco users will reach almost 1.9 billion. Tobacco use in Pakistan is increasing, especially in women and children (2). Studies carried out in India and Bangladesh found that tobacco smoking is more common in men who are the main economic support of their families. Tobacco use is usually in the form of cigarettes, cheroots, bidi and smokeless tobacco (3, 4). Different studies have been carried out suggestive of an association between cigarette smoking and cataract (5-8). Nuclear cataract is the most common type in South Asia and is found to be associated with cigarette smoking (9).

Cataract is the most common cause of blindness in the world, especially in under-developed countries due to lack of awareness and health facilities (8). Pakistan National Blindness and Visual Impairment Survey estimated that prevalence of blindness due to cataract was 1.8% in women and 1.67% in men in 2007, which is estimated to be almost double by the year 2020 (10).

Cigarette smoke contains toxic substances such as toxic aldehydes, aromatic hydrocarbons, nitrosamines, benzopyrenes, metals and free radicals (11-13). Each single puff of cigarette smoke constitutes 1014 free radicals of low molecular weight (12, 13). Since free radicals are unstable, they react with other important cellular molecules and acquire stability leading to cell malfunction (14).

Any damage to lens cells and/or proteins can result in lens opacity (15). Human eye lens contains high concentration of crystalline proteins which are water soluble in nature. Crystalline protein prevents aggregation which is desired for cataract formation (16). Mature fiber cells predominant in the nuclear region lack mitochondria (17). Metabolites and nutrients reach fiber cells via gap junctions (18). Alteration in gap junctions which are mostly composed of connexin 46 and connexin 50 effect lens survival (15).

Oxygen is important for all life forms except anaerobic micro-organisms. It acts as an electron acceptor but it also produces oxidative stress. Free radicals produced due to oxidative stress react with other molecules. The process may continue and lead to cell malfunction (14). Reactive oxygen species are also important in maturation of cellular structure

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at low to moderate level. Patients unable to produce superoxide anion radicals are susceptible of developing chronic infections (19). Reactive oxygen species also play a role in cellular signaling (20). Antioxidants help in removing free radicals and try to keep a balance between oxidants and antioxidants and maintain cell functions (21-23).

Among antioxidant enzymes, Superoxide Dismutase, Catalase and Glutathione peroxidase can be referred to as the important ones (24). Other important antioxidants like Glutathione can exist in aqueous humor (24). Glutathione produced in cortex reaches the lens nucleus by intercellular pathways and glutathione concentration is maintained in reduced form in order to prevent cross-linking (25). Molecules up to 10 KDa including antioxidants can pass through gap junctions into the intercellular passage (26). Diffusion pathways are very important for maintenance of lens transparency (27). Oxidative stress causes disturbances in intercellular communication because of damage to lens gap junctions by damaging the proteins involved in their formation, i.e., the connexin CX 43, CX 46 and CX 50 (26, 28). Intercellular communication is regulated by gating mechanism of connexin hemmichannels. Metal ions like cadmium produce local narrowing and closure of large aqueous connexin pore due to affinity of sulfhydryl groups of hemmichannels opening (29). Decrease in glutathione level in lenticular cells due to lack of diffusion through gap junctions increases cell susceptibility to toxic effects of cadmium which produces reactive oxygen species in cells (26). Oxidative stress can cause non-enzymatic post-translational modifications of lens proteins in lens which results in increased chances of oxidation and cross-linking, accumulation of fluorescent chromophores and increased scattering of light (30). Binding to Internal metal binding site of the Permeability Transition Pore, lead may open them initiating the cascade of cytochrome-c resulting in apoptosis of cell (31). Metals produce HO radicals which can be involved in protein modifications as evident by presence of hydroxylated aminoacids in crystalline of cataractous lenses (32).

The aim of the study was to look for the mechanism associated with metal deposition due to cigarette smoking and its effect on antioxidant enzymes in lenses of smokers with cataract.

## METHODS

The study design was cross-sectional. Twenty cataract patients were randomly selected according to selection criteria from the Out Patient Department of Layton Rahmatulla Benevolent Trust Hospital (LRBT), Karachi, after getting permission from ethical committee. Layton Rahmatulla Benevolent Trust Hospital (LRBT) hospital is a well reputed charity hospital and is visited by patients from Karachi and Sindh. Patients suffering from nuclear cataract grade II according to LOCS II (Lens Opacity Classification), aged 40 to 60 years, smoking more than one pack, i.e., 20 cigarettes per day for the last 20 years, and no break up in smoking for more than one month were selected. They were excluded if they had history of diabetes, hypertension, glaucoma, trauma to eye or/and alcoholism and history of viral infections like rubella and so on.

Random sampling was done. Every third patient visiting for visual impairment was interviewed and if they had including criteria they were selected. Ten smoker patients and 10 non-smoker patients were selected. Informed consent was taken from all patients after a careful explanation of the aims of the study. Extra capsular cataract extraction surgical procedure was done on these patients. After acquisition, lens samples were kept in individual glass bottles already cleaned by nitric acid and deionized water and then deeply frozen until analysis. Each lens was cut into two equal halves, one half for metal analysis and the other half for enzyme analysis.

**Metal Analysis:** One half of the lens was dissolved in concentrated nitric acid for decomposition and left for 30 minutes. Then it was placed on sand bath at 140° C to complete the digestion of the lens. The dried decomposed material was then dissolved in deionized water and then concentration of lead, cadmium and copper were determined by Hitachi Atomic Absorption Spectrophotometer Z-8000 with Zeeman Effect background correction, equipped with graphite furnace, a microprocessor and built-in printer.

**Antioxidant Analysis:** One half of the lens was quickly homogenized in 1.5 ml of 1.15% KCl in an ice bath. The soluble protein was washed out until no protein was detected by absorbance at 280 nm and then analyzed for Glutathione peroxidase and Superoxide dismutase by kit method using micro plate reader.

All the tests were done in Pakistan Council of Scientific and Industrial Research. A bivariate Pearson Correlation Analysis was done in SPSS 6.0 (Statistical Package for the Social Sciences) to establish whether significant difference in enzyme concentration exists between the Smoking and Non-Smoking Group.

## RESULTS

This study found a higher Concentration of trace metals (Cu, Cd, Pb) in smokers as compared to non-smokers as seen in table 1. The Tendency of metal deposition was highest in copper then lead & then Cadmium.

The effect of smoking on the concentration of antioxidant enzymes (Glutathione peroxidase & Superoxide dismutase) in sampled lenses are presented in table 2 and table 3. It is shown in table 2 that the Concentration of Glutathione peroxidase is almost half in smokers as compared to non-smokers. There was a mean difference of 1.727µmol/mg of Glutathione peroxidase between non-smokers & smokers.

It was also observed that the level of superoxide dismutase Concentration among (almost one fifth) smokers group was found to be even significantly less than the non-smokers as

**Table 1.** Descriptive Statistics of Trace Metal Concentration among the 20 patient studied

Groups	Lead mean (SE) µ g/gm.	Cadmium mean (SE) µ g/gm	Copper mean (SE) µ g/gm
Smoker (n=10)	2.51 ± 0.237	0.15 ± 0.04	2.79 ± 0.876
Non-Smoker (n=10)	0.57 ± 0.07	0.01 ± 0.003	0.90 ± 0.0885

**Table 2.** Descriptive Statistics of Glutathione Peroxidase

umol of GSH consumed/mg of protein	N	Minimum	Maximum	Mean	Std. Error
(Overall Group)	20	1.02	4.06	2.2815	0.22
Smokers	10	1.02	2.05	1.41800	0.11
Non-Smokers	10	2.23	4.06	3.14500	0.167
Difference between Mean of Smokers and Non-Smokers				1.727	

**Table 3.** Descriptive Statistics of Superoxide Dismutase

umol of SOD consumed/mg of protein	N	Minimum	Maximum	Mean	Std. Error
(Overall Group)	20	.26	2.60	1.183	0.193
Smokers	10	.26	.58	.379	0.662
Non-Smokers	10	1.43	2.60	1.987	.114
Difference between Mean of Smokers and Non-Smokers				1.608	

shown in table 3. There was a mean difference of 1.608 $\mu$ mol/mg between non-smokers & smokers.

This study found an negative correlation between the Glutathione peroxidase levels (as estimate by the GSH consumed / mg of protein) and each of the three metals (Pb, Cd , Cu). The negative correlation was highest between Glutathione peroxidase with Lead. Similarly, the Concentration of Superoxide dismutase had a negative correlation with the concentration of metals especially lead as shown in table 4.

## DISCUSSION

Studies have been carried out in the past which have indicated a potential role of increased oxidative stress due to decrease antioxidants in the pathogenesis of cataract (27-35). The level of reactive oxygen species varies depending on its production and removal by antioxidant systems. Various antioxidants are present in cells which prevent or repair the damage due to reactive oxygen species. For the survival of oxygen utilizing cells three antioxidant enzymes are essential which are Glutathione peroxidase, Superoxide dismutase and Catalase (36). Superoxide radical converts into hydrogen peroxide and molecular oxygen by the action of Superoxide dismutase. The hydrogen peroxide converts into water by the action of Peroxidases and Catalase. Catalase converts hydrogen peroxide into water and oxygen. In this study we determined the level of metals and antioxidant enzymes in cataract lenses of smokers and non-smokers to see the effect of smoking on progression of cataractogenesis. Higher levels of copper, cadmium and lead were found in smoker's lenses than non-smoker lenses (Table 1). Glutathione peroxidase and superoxide dismutase levels were significantly lower in smoker lenses than non-smoker lenses indicating that smoking leads to increased deposition of metals in lenses causing impairment of antioxidant enzymes (Table 2 and 3), which resulted in damage to lens structure and function. The current study showed a negative correlation of Increased

Metal Concentration (Lead, Copper, Cadmium) with antioxidant enzymes concentration (Table 4). Glutathione peroxidase is a well-known selenoprotein present in most human tissues. It metabolizes hydrogen peroxides and lipid hydroperoxides (37). Decreased activity of glutathione peroxidase has been reported to cause hemolytic anemia in premature infants (38). Heavy metals are reported to affect glutathione peroxidase activity (39). Metals combine with thiol group of protein and enzymes. This results in impairment of antioxidant capability of reduced glutathione. This enzyme keeps thiol group of protein in reduced form essential for transparency of lens (40). Ahmed et al. (2014) analyzed copper, lead and cadmium levels in lenses of 75 smokers and 25 non-smokers in Karachi, Pakistan, and found significant higher levels of metals in lenses of smokers than non-smoker lenses supportive of the results of this study. The study also found that the number of cigarettes smoked affects the process of cataract by accelerating it. The heavy smokers developed cataract earlier, as smoking affects the protein denaturation by producing radicals (37). Antioxidant enzymes are affected by the number of cigarettes smoked and also duration of smoking (41). X-Wang et al. (2014) found that antioxidant enzymes Superoxide Dismutase, Catalase and Glutathione Peroxidase decreased significantly with progression of cataract (42). Tang et al. (2013) studied the genetic variation due to smoking and its effect on lung function. They observed variants in superoxide dismutase (SOD3) resulted in decrease in lung function by observing FEV1 and FEV1/FV. This study helped to understand and develop future strategies for loss of organ function associated with cellular destruction and enzymatic changes (43). Bormusov et al. (2013) in their study looked for the role of antioxidants in prevention of tobacco smoke effect on cultured bovine lenses. They found that the spaces between lens epithelial cells were increased. Some cells were without nuclei and membrane. Cellular organization was changed showing hyperplasia, hypertrophy and change in nucleic acid

**Table 4.** Correlation Values of Glutathione Peroxidase and Superoxide Dismutase with Lead, Cadmium and Copper

Pearson Correlation	N	Pb ug/gm	Cd ug/gm	Cu ug/gm
Umol of GSH consumed/mg of protein	20	-.876	-.484	-.482
Umol of SOD consumed/mg of protein	20	-.867	-.578	-.461

density. With the help of fluorescent staining (Dihydrofluorescein Diacetate), reactive oxygen species in epithelium was detected and followed up. They found that desferoxamine partially prevented the cell damage while N-acetylcarnosine (NAC) totally prevented the damage (44). Chang et al. (2013) in their study analyzed the products of oxidative stress activity of antioxidant enzymes in healthy control subjects and patients with age-related cataracts. They found significantly decreased activity of superoxide dismutase in cataract patient serum ( $97 \pm 13.56$  U/ml) as compared to healthy individuals ( $103.47 \pm 1818.97$  U/ml). Similarly, the level of glutathione peroxidase was significantly decreased in cataract patients ( $135.75 \pm 20.21$   $\mu$ mol/l) as compared to healthy individuals ( $147.90 \pm 90$   $\mu$ mol/l). The level of oxidation degradation products malondialdehyde (MDA), 4-hydroxynoneal (4-HNE) and conjugated diene (CD) was significantly higher in cataract patients than healthy controls. The result shows the important role of oxidative stress in cataractogenesis (45). Julius et al. (2014) studied the effect of cigarette smoking leading to oxidative stress in human. They collected blood samples from smokers and nonsmokers and analyzed for lipid hydroperoxide, superoxide dismutase and catalase. They found significantly low level ( $4.54 \pm 0.84$  unit/mg) of superoxide dismutase in smokers than nonsmokers ( $9.32 \pm 0.70$  unit/mg). Catalase level was also significantly lower in smokers ( $86.03$  (86.03y low level ( $4.54 \pm 0.84$  unit/mg) of superoxide dismutase in smokers than nonsmokers and a  $0 \pm 3.6$  mg/dl in blood) in nonsmokers than smokers ( $143 \pm 56.65$  mg/dl in blood) (46). The result indicated that oxidative stress was produced due to smoking and it affected antioxidant enzymes and cell integrity. Cigarette smoke has shown to cause skeletal muscle problems by producing inflammation, metabolic disturbances and oxidative stress. It also causes enhanced expression of genes related to atrophy.

#### LIMITATION

Number of participants could have been more but it was not possible because of financial limitations. It resulted in inability to further categorize the samples according to frequency of cigarette smoking.

#### CONCLUSION

Prevention or delay in developing cataract by use of chelators and antioxidants topical and systemic use could be helpful. Larger and prospective clinical studies and trials in this respect are needed.

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