ejpmr, 2017,4(9), 176-183

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EFFECT OF CIGARETTE SMOKING ON WEIGHT OF HUMAN LENS

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Article Received on 17/07/2017 **Article Revised on 07/08/2017** **Article Accepted on 27/08/2017**

ABSTRACT

Background: The growth of lens of eye takes place throughout life due to addition of new cells from the single layer of cuboidal cells beneath the capsule and thus may remain exposed to influences like oxidative stress for longer period. Cigarette smoking leads to deposition of metals in smokers and even in their lenses, known to cause oxidative stress. The aim of study was to see the association of metal deposition due to cigarette smoking on weight/growth of lens. **Materials & Methods:** Total 100 patients having nuclear cataract including smokers and non-smokers were randomly selected from OPD of LRBT Hospital. After surgical extraction (ECCE) lenses were weighed by scientific balance and then copper, cadmium and lead concentrations were determined by Atomic Absorption Spectrophotometer. **Result:** Weight of Lens was found to be significantly lower in Smokers than Non-Smokers. Also Negative Correlation between Concentration of Metals and Weight of Lens was observed. **Conclusion:** The study is indicative of how smoking (air pollution) affects the cellular integrity and viability of lens of human eye. It emphasizes the importance of preventive measures by identifying the relationship of environmental pollution and increasing cases of different organ failures to improve the general health and economics of people.

KEYWORDS: Smoking, Lens, Weight, Oxidative, Metals.

INTRODUCTION

The aim of study was to see the effect of cigarette smoking on weight of lens in smokers. As the growth of lens of human eye takes place throughout life by addition of new cells, any disturbances in these cells can cause impairment in growth/weight of lens leading to disorders like cataract and presbyopia. The study was conducted in Karachi, Pakistan. Pakistan is situated in Eastern Mediterranean Region (EMRO) of WHO and is a part of developing countries. Pakistan is also affected by the tobacco epidemic and the prevalence of tobacco use in women is increasing.[1] (Addiction.2001 Dec; 96 (12):1847-54).

Cigarette smoking is known to cause deposition of metals like cadmium, lead and copper in human lens leading to early cataract[2,3] (ahmad A et. al 2014, Cekic O 1998).

Lens of human eye has aqueous humor in front and vitreous humor behind. Aqueous humor is formed from plasma by ciliary network of ciliary processes. It provides substrates (glucose, oxygen, electrolytes) for metabolic needs of avascular lens &cornea along with stability of structures of eye.[4] (Sires, 1997).

Crystalline Lens is composed of four layers from outside to inside (capsule, sub capsular epithelium, cortex and nucleus). Two types of cells are found in vertebrate lens. Lens epithelium is present anteriorly and the lens fiber cells posteriorly[5,6] (Bhat, 2001, Lovicu & Robinson, 2004). Beneath the capsule a single layer of cuboidal epithelial cells forms the anterior surface. These cells divide near the equator and then these divided cells elongate and convert into fiber cells. Shape of thesefiber cells are flattened hexagonal and they get arranged in such an orderly manner that the spaces between the cells are smaller than the wavelength of light.[7] (Paul et al; 2010). Because the lens fiber cells are compactly placed in the center of lens, they lose water. This is because of the water bound to protein is released and then leaves out of the cell[8] (Lahm et al, 1985). Mitochondria are abundant in the lens but only within the epithelium and differentiating fibers, mature fibers in the center of the

lens lack mitochondria[9] (Huang et al; 2008). Nutrients and metabolites reach these fiber cells which lack mitochondria in the center of the lens via jap junctions[10] (Goodenough et al, 1980).

Each mature fiber cell is arranged anteroposteriorly. Lens prevents light scattering mainly in three ways by keeping the lens crystallines in soluble form, controlling the volume of its cells and preventing extra cellular space dilatation[7] (Paul et al, 2010).The lens has no blood vessels and is multicellular. Aqueous humor is a clear fluid and functions like blood for the avascular structures i.e Lens and cornea.[11,12] (Goldmann1950, Ascher 1954).

In most of the ocular diseases including cataract, oxidative stress and metabolites of polyunsaturated fatty acids play a major role.[13,14,15] (Njie et al, 2013 Cadenas, 2000 Wickens, 2001).Studies have been carried out suggesting association between oxidative stress and cataract formation.[16,17,18,19,20,21,22] (Ottonelloetal, 2000; Guletal, 2008; Fernandez, 2008; Rumyantseveetal, 2008; Yildirim et al, 2009; Li et al, 2009; Ho et al, 2010).

Carper et al (1992)[23] in a study exposed human lens epithelial cell line to oxidative stress by exposing to 200 micromole hydrogen peroxide and found changes in expression of certain specific genes which have important role in respiration of cell and processing of mRNA and peptide.

Superficial cells of lens are metabolically active & substrates from these cells can diffuse to mature nuclear fiber cells via gap junctions.[24] (David et al, 2010). Berthoud et al(2009)[25] have explained that oxidative stress leads to disturbances in intercellular communication due to damage to lens gap junctions. It thus impairs transport of nutrients, oxygen and metabolites to reach nuclear region and also excretion of waste resulting in disturbances in cellular function and even apoptosis.

Connexin hemi channels openings have regulated closure and opening mechanisms known as loop gating which prevent cell damage from excessive ion flux and metabolic substances. Cadmium (Cd+2) at a very low concentration (50-100nM) leads to closure of hemichannels. It is because of sulfhydryl groups of hemichannel opening is being accessed by metal ions producing local narrowing and closure of large aqueous connexin pore due to higher affinity[26] (Mathieu et al, 2010). Oxidative stress can effect lens gap junctions & the proteins involved in their formation i.e. the connexin (CX43,CX46&CX50) thus it may lead to altered intercellular communication.[27] (Viviana et al,2009).

GSH is formed & then reduced in cortex of lens (peripheral part of lens). The lens nucleus has little capability of reducing glutathione so reduced glutathione diffuses from periphery to its center. Thus the diffusion pathways are vital for maintenance of lens transparency and growth.[24] (David et al, 2010). Growth of lens continues throughout life. In about fourth decade transport of glutathione into the nucleus gets restricted. It affects the lens transparency and flexibility.[28] (Michael et al, 2010). As the growth of lens takes place throughout life the lens core may remain exposed to influences like oxidative stress for longer period. Non enzymatic post translational modifications of lens proteins take place which results in increased chances of oxidation & cross linking, accumulation of fluorescent chromophores & increased scattering of light.[29] (Michael et al, 2011). Decrease in glutathione (GSH) 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.[27] (Viviana et al, 2009).

Metals decrease connexin protein levels and gap junctional communication. Further studies to develop medicines which may improve hemichannel signaling and gap junction could be promising[26] (Mathieu et al, 2010).

Exposure of lead causes accumulation of s-amino levulinic acid (ALA) in cells producing reactive oxygen species and oxidative damage resulting in decrease antioxidant defense mechanism and lipid peroxidation.[30,31] (Guillermo et al, 2003; Flora et al, 2007). It is also responsible for genotoxic effects[32] (Fuchs et al, 2000). Lead produces decrease and altered activity of antioxidant enzymes like catalase, glutathione peroxidase, superoxide dismutase glucose-6 phosphate dehydrogenase, G-6-P-D is very important for those cells which do not have mitochondria[33,34,35] (Ito et al, 1985; Sugawara et al, 1991; Chiba et al, 1996). Lead increases the intracellular calcium and causes mitochondrial depolarization, swelling & release of cytochrome-C resulting in cellular apoptosis[36] (Flora et al, 2007). It has been suggested that lead may bind to internal metal (me +2) binding site of the permeability transition pore (PTP) & then open the permeability transition pore initialing the cascade of cytochrome – C resulting in apoptosis of cell.

Cadmium gets deposited in lens and decreases the viability of lens epithelial cells. This is also indicated by increase in apoptic cells. LDH (lactic dehydrogenase) releases from HLECs which is related to the amount of cadmium. Cadmium increases oxidative stress, peroxidation of lipids and MAPK pathway activation. α-Tocopherol and N-acetylcysteine decreases toxicity of cadmium in HLECs[37] (Nilesh et al, 2010).

Intracellular reactive oxygen species are mainly produced in mitochondria and this makes the mitochondria more vulnerable to oxidative stress.[38] (Szeto et al, 2006).

Stuart et al (2010)[39] described the importance of mitochondria in disorders of eye due to aging and genetic

transfer. Mitochondria have the main role in providing energy to cell and its survival. Cumulative damage to mitochondria can occur due to oxidative damage. Mitochondrial DNA is not as stable as nuclear genome because it lacks some of the repair mechanism (Nucleotide Excision Repair) thus resulting in mitochondrial impairment.

Transforming growth factor – beta (TGF beta) causes abnormal changes in epithelial cells of lens like bleb formation in cell surface, lens capsule wrinkling, apoptic cell loss and inability to express PaX6. Glutathione and catalase are important antioxidant system of lens. They not only protect the lens epithelium from damaging effects of reactive oxygen species but also prevent the epithelial changes due to TGF beta[40] (Coral et al, 2009). Mutations in PaX6, PaX2 and SOX2 have been identified as a cause of micropthalmia.[41] (RaggeNK et al, 2005).

At the end of chromosomes telomeres are present. During each replication they shorten & this mechanism helps in mitotic counting. Oxidative stress & antioxidant defense capability of lens influences the rate of telomere shortening in human lens epithelial cells. Cadmium is known to cause its deleterious effects by deactivating DNA repair activity[42] (McMurray et al, 2003).

Cadmium inhibits only mismatch mode of repair. Cadmium induced inhibition of MMR causes about 20-50% of DNA mismatch unrepaired. Inhibition of MMR lead to propagation of cellular errors, thus in this way toxic effects of cadmium can be amplified in cells by creating mutations in genes that induces further faulty functions[43] (Jin et al, 2003).

MATERIALS AND METHODS

The study was conducted in southern part of Pakistan at Karachi. The study design was cross-sectional. Patients were selected from the Out Patient Department (OPD) of Layton Rahmatullah Benevolent Trust (LRBT) Hospital after the approval of ethical committee. The people of the province of Sindh and even Baluchistan visit the facility for consultation for being a well reputed charitable organization. Every third patient visiting the OPD for visual problem went through General physical and ophthalmic examination and if they were within selection criteria, they were explained about the study and written consent was taken by assuring them confidentiality. Patients having nuclear cataract grade II according to LOCS II, with history of smoking for the last 20 years without having any breakup for more than four weeks and 40-60 years of age were selected. Those who were having hypertension, diabetes, glaucoma and history of rubella, trauma of eye and alcoholism were excluded.

100 patients were selected for the study by Random Sampling Method. The selection was based on a detailed interview using validated questionnaire asking about their personal data, health status, home and work places environment and their smoking habit. 75 patients selected were smokers having nuclear cataract. They were categorized into two groups according to number of cigarettes smoked/day. Group 1 (half to one pack/10-20 cigarettes/day) and Group 2 (more than one pack/ > 20 cigarettes/day). 25 patients having nuclear cataract were selected as controls having no history of cigarette smoking or tobacco chewing.

The patients went through the surgical procedure of extra capsular cataract extraction (ECCE) and the acquired lens samples were then kept in individual labeled glass bottles. These glass bottles were priory cleaned by nitric acid and deionized water. The weight of the lenses were carefully recorded by scientific balance & then stored at -20C till metal analysis.

For metal analysis 3ml of conc. nitric acid (65%) was added in the Lens sample bottle for digestion of lens material and kept at ambient temperature for 30 minutes. Then it was transferred into a 25ml beaker. To remove any leftover in sample bottle 2ml of conc. nitric acid was added in sample bottle & then it was transferred into the beaker. To complete the digestion the beaker was placed on sand bath and temperature was gradually increased to 140C till complete digestion and the sample was almost dry. To cool the decomposed material beaker was kept at ambient temperature. The decomposed material was dissolved in 5ml deionized water and then transferred into 25ml calibrated volumetric flask. 10ml deionized water was added twice in the beaker to rinse it & then transferred to the 25ml volumetric flask. It was then used for metal analysis by Atomic Absorption Spectrophotometry. Blank sample was prepared similarity to eliminate any possible contamination during digestion process.

Cadmium, Lead and Copper Concentration were measured in the prepared samples of lenses. Hitachi Atomic Absorption Spectrophotometer (Z-8000) with Zeeman effect background correction was used for metal analysis which was equipped with a graphite furnace, a microprocessor and a built in printer.

RESULTS

A statistical association between concentration of metal (Lead, Cadmium and Copper) and smokers/non-smokers was studied in an earlier study (Ahmad et al 2014)[2], in which it was concluded that a statistically significant association exists between concentration of metal (Lead, Cadmium and Copper) in human lens and smoking.

Table-1 shows the descriptive statistics of the 100 patients that were studied. An overall mean of 0.3627g with an S.D of 0.1322 was observed among the patients. The minimum weight of lens was found to be 0.1343gm; while the maximum weight was found to be 0.75gm.

Table 1 (Descriptive Statistics among Patients studied).

	N	Minimum	Maximum	Mean	Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Statistic
Weight of Lens gm	100	.1343	.7584	.362702	.1322550
Valid N (listwise)	100				

Table-2 shows a comparative statistics between smokers and non-smokers. Among the smokers group a mean Len's weight of 0.327gm with an S.D of 0.107 was observed. The maximum weight of the lens among the smokers was found to be 0.6126gm while minimum weight was found to be 0.1343gms. In comparison among the non-smokers group the mean weight of the Lens was found to be 0.468gm with an S.D of 0.145gms. The maximum weight of the Lens among the non-smokers was found to be 0.758gm while minimum weight of lens was found to 0.26gm.

Table 2 (Comparison between Smokers and Non-Smokers).

	N	Minimum	Maximum	Mean	Std. Deviation
Smokers	75	.1343	.6126	.327280	.1070269
Non-Smokers	25	.2600	.7584	.468968	.1454276

An Independent Sample T-test was conducted, to ascertain whether the two groups are significantly different. The Levene's test for Homogeneity of Variance calculated a p-value of 0.047, which is a little less than 0.05. The p-value calculated in both cases was found to be 0.00 again less than 0.05 for a 95% Confidence Interval, meaning that our Ho Hypothesis could be rejected in favor of Alternate Hypothesis Ha i.e. Mean Weight of Lens in Smokers is not equal to Mean Weight of Lens in Non-Smokers.

Thus we could say with reasonable confidence that difference in two groups is statistically significant. Detailed Results calculated from SPSS are given in Table-3.

Table 3 (Result of Independent Sample T-test between Smokers and Non-Smokers).

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Weight of Lens gm	Equal variances assumed	4.048	.047	-5.217	98	.000	-.1416880	.0271577	-.1955816	-.0877944
	Equal variances not assumed			-4.483	33.098	.000	-.1416880	.0316022	-.2059759	-.0774001

In order to establish the association between metal concentration and weight of lens, Pearson.

Correlation test was done (Detailed Results are given in Table-4). Following are the results

1) There was a negative correlation between Weight of lens and Lead Concentration, r = -.344, p = < .05, with a R^2 = .118
2) There was a negative correlation between Weight of lens and Copper Concentration, r = -.234, p = < .05, with a R^2 = .054
3) There was a negative correlation between Weight of lens and Cadmium Concentration, r = -.344, p = < .05, with a R^2 = .118

Table 4 (Result of Pearson Correlation between Weight of Lens and Metal Concentration).

		Pbug/gm	Weight of Lens gm	Cu ug/gm	Cd ug/gm
Weight of Lens gm	Pearson Correlation	**-.344****	**1**	**-.234***	**-.344****
	Sig. (2-tailed)	**.000**		**.019**	**.000**
	N	**100**	**100**	**100**	**100**

DISCUSSION

Metals present in cigarettes are responsible for producing reactive oxygen species.[44,45] (Garner et al, 1999; Santon et al, 2004). As metals have a long half-life deposition in tissues occur[46] (Grubb et al, 1985). The radicals produced can cause peroxidation of Lipids, biomolecules degradation and breakup of DNA strand[47] (Anselm et al, 2013). Cumulative damage to mitochondria can occur due to oxidative damage which is important for providing energy and cell survival[48] (Jarrett et al. 2010).

Metals combine with proteins and enzymes having thiol group impairing their function directly and also indirectly as antioxidant enzymes such as reduced Glutathione, which keeps thiol groups in reduced form responsible for transparency of lens[49] (Ganea, 2006). Decrease level of antioxidant enzymes lead to oxidative damage to lens epithelial cells even at low concentration affecting Na/K ATPase, cytoskeletal proteins & membrane permeability[50] (Giblin, 2000) and even lead to apoptosis[51] (Kalariyaa et al, 2010).

Song et al (2012)[52] in their in vitro study on cultured lens epithelial cells looking for effects of cadmium found that pathological changes developed suggestive of cellular toxicity & damage. The damage to epithelial cells could impair the only regenerative layer of epithelial cells of lens resulting in decrease in number of lens cells. The evidence is supportive of my result as the weight of lens in smokers having higher concentration of metals was less than that in non-smokers.

Graziano et al (2014)[53] found that tobacco exposure affected almost all macromolecules related to structure and/or function of cell due to oxidative damage. This study is also supportive of the result of my study as the onset of cataract is earlier in smokers and weight of lens is also lesser than that in non-smokers.

Julius et al (2014)[54] studied effect of cigarette smoking leading to oxidative stress in human. They collected blood samples from smokers and nonsmokers and analyzed for lipid hydroperoxide.

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.

The result of this study also shows that the weight of lens in smokers was statistically found to be less than that in non-smokers. The weights of lenses in both groups were also having relationship with frequency of smoking. The mean weight of lenses was in decreasing order from non-smokers, moderate smokers and heavy smokers.

Spencer (1976)[55] in his article described that the weight of human lens increases with age (L=1.32A+141 L=Wt. of lens in mg A= age in years).

Augusteyn (2007)[56] studied the growth of human eye lens on 1100 human lenses. The samples were taken from persons from 6 months to 99 years of age. He found that there is a rapid growth period during prenatal period which almost stopped immediately after birth and then there was a period of linear growth when the weight of lens increased by adding approximately 1.38 mg/year.

Mohamed et al (2012)[57] studied the growth of lens in Indian population and found that the growth of lenses were in two phases similar to as observed by Augusteyn. It increased linearly at 1.24 mg/year.

Rom et al (2012)[58] in their review article about studies done for effects of cigarette smoke (CS) on muscle catabolism revealed that studies have identified that cigarette smoke produced muscle atrophy due to enhanced expression of genes related to atrophy. CS produced inflammation, metabolic disturbances and oxidative stress.

Lens has only a single layer of cuboidal epithelial cells beneath the lens capsule. Only In this layer division and replication of cells take place mainly in equatorial region providing new cells which take the shape of elongated, flattened, hexagonal and closely packed fiber cells. These cells migrate towards nuclear region taking the function of refraction of light[9,59] (Huang et al, 2008; Donaldson et al, 2010).

Metals (cadmium) get deposited in lens cells decreasing their viability, increasing apoptic cells. These changes increase with increase in concentration of metals[60,61] (Drager et al, 1987; Neal et al, 2010). Even expression of certain gene changes which have important role inrespiration of cells and processing of mRNA and peptide can be affected by oxidative stress produced by metals[62] (Carper et al, 1992). As heavy metals compete with other essential metals for binding sites[60] (Drager, 1987) even replacing already bound metals[63] (Jamall et al, 1989) they compromise cellular nutrients and metabolites. They impart long lasting toxicity by having long half-life in cells[64] (Sarna et al, 1980).

All these above effects lead to impaired regeneration of new cells and even death of existing cells so they may be responsible for decrease in weight of lens in smokers.

The new fiber cells which lack mitochondria depend on gap junctions for nutrients and metabolites[10] (Goodenough et al. 1980). In lens a complex network of gap junctions exists mainly comprising of connexin 46 and connexin 50, which are essential for normal survival of lens[65,66] (Goodenough 1979; Beyer, 1993). They have important role in maturation of lens fiber cells and their elongation and also for transportation of metabolites,

ions and water in lens[67] (Gong et al, 2007). These intercellular pathways also transfer antioxidant enzymes produced in single layer of cuboidal cells (Glutathione) to nuclear region[59] (Donaldson et al, 2010).

As metals decrease the number of gap junctions in each cell, decreases their function[68] (Jeong et al,2001) closing the loop gates of them at an even low concentration, producing local narrowing and closure of connexin pores[69] (Vinken et al, 2012) affecting the nutrition and metabolism of lens cell compromising cell viability.

All of the above effects may lead to decrease in growth and weight of lens.

CONCLUSION

The study is indicative of how smoking (air pollution) affects the cellular integrity and viability of lens of human eye. It emphasizes the importance of preventive measures by identifying the relationship of environmental pollution and increasing cases of different organ failures to improve the general health and economics of people.

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