Original Article :

Aqueous Extract Analysis of Lens Epithelial Cells: Unraveling the Link between Oxidative DNA Damage and Malondialdehyde in Senile Cataract.

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Received on Nov 10, 2023, accepted for publication on Jan 03, 2024, dx.doi.org/10.5455/mjhs.2024.03.009

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Abstract

Background and Aims:
The purpose of this study was to examine the level of oxidative stress in people with cataracts, based on previous studies that indicated its role in causing DNA damage and cataract formation. The study also sought to determine the correlation between the degree of DNA damage and the concentration of malondialdehyde, a marker of lipid peroxidation, in patients with senile cataracts.

Methods:
The study involved 30 senile cataract patients aged 50-80 years and 12 cadaveric donors of corneal tissue who died in road accidents. They were selected from the ophthalmology ward of Acharya Vinoba Bhave Hospital. The aim was to measure DNA damage in human lens epithelial cells (HLECs) by Comet assay and malondialdehyde levels in the aqueous extract of HLECs.

Results:
The study found that the oxidative DNA damage level of human lens epithelial cells is not correlated with the malondialdehyde levels in the aqueous extract of HLECs in senile cataract patients.

Conclusions:
The study did not support the hypothesis that oxidative stress is a major factor in the development of cataracts. The results suggest that other mechanisms, such as genetic factors, environmental factors may be more relevant in the pathogenesis of cataracts.

Keywords:
Cataract, DNA damage, malondialdehyde.

Introduction

"Cataract is a pathological condition characterized by the loss of lens transparency, resulting in impaired vision . [1] It is the main cause of blindness globally and can affect one or both eyes. The most im-

portant factor that affects the development of cataracts is aging, as it causes changes in the lens of the eye. Other factors such as chronic exposure to ultraviolet radiation, smoking, and diabetes mellitus also have an impact on cataract development. [2] Free radicals are constantly produced within the body, mainly through the activity of mitochondrial electron transport chain enzymes, such as xanthine oxidase and aldehyde oxidase.[3] In addition, various processes such as inflammation, xenobiotic metabolism (the breakdown of foreign substances), and exposure to high levels of oxygen (hyperoxia) produce free radicals. . [4] These free radicals can induce oxidative stress, which damages cellular components and may contribute to various health conditions. The free-radical theory of aging postulates that reactive oxygen species (ROS) cause oxidative damage throughout an individual's life. Over time, the accumulated and potentially increasing amount of this damage is thought to cause various dysfunctions and pathologies observed in the normal aging process. In essence, the theory implies that the continuous exposure to ROS results in cellular and molecular damage, which leads to age-related changes and age-associated health issues. The generation of reactive oxygen species (ROS) through photosensitizing mechanisms can be initiated by exposure to light. Tissues, such as the lens, regenerate antioxidants slowly, making it more prone to the accumulation of oxidative damage over time. The lens has innate defense systems that can protect it from oxidative stress, but its protein turnover, especially in the nuclear region, is low. This low turnover may impair the lens's capacity to effectively repair and eliminate oxidized proteins.[5] Previous studies have demonstrated that lens proteins exposed to ultraviolet radiation (UVR), such as sunlight, with or without aromatic amino acids, can produce modified proteins. These proteins have features like insolubility, pigmentation, and fluorescence. These features are attributed to photochemical reactions and oxidative damage induced by the interaction of UVR with lens proteins, particularly those with aromatic amino acids. The accumulation of these altered proteins can cause lens opacity and cataract formation over time. Since crystallins and other proteins in the lens fibers have minimal regeneration, they have to persist and function for a person's lifespan. Therefore, the lens needs more protection from oxidative stress than many other tissues. . [6] The buildup of oxidative damage in the lens proteins can result in cataracts and compromise the lens's transparency and function. Hence, mechanisms to prevent and counteract oxidative damage are essential for preserving the

lens's clarity and function. As an individual ages, the accumulation of abnormally high levels of reactive oxygen species (ROS) can occur due to a combination of overproduction and inadequate removal of these molecules. ROS can cause damage to various cellular components, including proteins, nucleic acids (such as DNA and RNA), and membrane lipids. This oxidative damage can lead to cellular dysfunction and contribute to the development of age-related health issues and diseases. The process of oxidative stress is thought to be one of the key factors in the aging process and the onset of age-related diseases. Maintaining a balance between ROS production and antioxidant defense mechanisms is essential for cellular health and overall well-being during the aging process. Lipid peroxidation is a process that occurs due to the oxidative damage caused by reactive oxygen species (ROS) on cell membrane lipids. As a result of this process, lipid peroxides are formed, which serve as indicators of oxidative damage in cells and tissues. These lipid peroxides are unstable and break down to form a complex array of compounds, including reactive carbonyl compounds. [7] Among these, malondialdehyde (MDA) is the most abundant and widely used as a biomarker for assessing oxidative damage in biological systems. Elevated levels of MDA and other lipid peroxidation products are often associated with oxidative stress and various diseases in humans. Lipid peroxidation is a process that generates reactive aldehydes, such as malondialdehyde (MDA), which can damage cellular components and impair their functions. Measuring MDA levels can reveal the degree of lipid peroxidation and its implications for cellular health and oxidative status. MDA levels are useful indicators of lipid peroxidation and oxidative stress in the pathogenesis of cataract.[8] DNA damage is another consequence of oxidative stress, and different types of DNA lesions can reflect different sources of oxidative insults. Reactive oxygen species (ROS) are among the main causes of DNA damage in human lens epithelial cells, which play a key role in maintaining lens homeostasis and preventing cataract formation . [9] The purpose of the mentioned study was to assess the percentage of DNA damage and malondialdehyde (MDA) levels in the lens epithelial cells of patients with cataracts compared to a control group. Furthermore, the study aimed to investigate whether there is a statistical correlation between oxidative DNA damage and malondialdehyde specifically in senile cataract patients.

Materials and Methods

Upon providing a comprehensive ex-

planation regarding the nature and potential implications of the study, informed consent was acquired from the participants. The Institutional Ethics Committee issued a letter of approval with reference number DMIMSU/IEC/2008-09/151, dated 30.05.2008.

Subject Selection

We collected capsulorhexis samples from 30 senile cataract patients aged 50-80 years who underwent cataract surgery at AVBRH ophthalmology ward. We used these samples to assess DNA damage and TAC levels in human lens epithelial cells by Comet assay. For malondialdehyde assays, we used 12 lenses from the eyes of healthy donors who had passed away in road traffic accidents and had given their consent for corneal transplantation.

Selection Criteria:

We excluded patients with type II diabetes mellitus, hypertension, trauma, steroid use, or any other systemic illness. We also excluded patients with traumatic or congenital cataracts. None of the subjects were chronic smokers or took multivitamin supplements such as vitamins A, C & E.

The study involved examining the eyes of patients who had senile cataract. The participants gave their ocular history. Professors from Our Institute's Ophthalmology Department confirmed the cataracts and their type before surgery.

Sample collection:

The patients received local anesthesia with 2% lignocaine. To remove the cloudy lens mass in cataract surgery, phacoemulsification is used. A tiny cut of about 2 millimetres is made near the cornea to reach the lens capsule. Then, a small round opening (about 5 millimetres across) is made in the capsule to access the lens fibres. The cloudy lens is broken up and taken out by an ultrasound device, leaving behind a capsular bag made of the back part of the capsule.The anterior capsule was extracted with Visco expression through the clear corneal incision and collected with forceps by an experienced surgeon. The sample was not handled or irrigated further to avoid harming the HLEC. The sample was immediately placed in an Essential Medium with 10% fetal bovine serum and transferred to the Research laboratory. A single rhexis for preservation was kept in a Minimal Essential Medium with 10% fetal bovine serum and incubated in a five percent carbon dioxide incubator at 37 °C . [10] The time-lapse from collection to processing was 15-20 min. The trypan blue exclusion test checked the viability of the human lens epithelial cells before the comet assay. [11] All samples were viable.

The human lens epithelial cells (HLECs)

were isolated from the capsule by mechanical shaking in 50 µl of Dulbecco's phosphate-buffered saline (DPBS, pH 7.2) for 15 min at 4 °C. The DNA damage in HLECs of senile cataract patients was assessed by Comet assay, following the protocol of Singh et al. [12] with some modifications: a) Glass slides were precoated with 75 µl of 1% high melting point agarose (HMPA) at 65 °C, covered with a coverslip and refrigerated for 10 min at 4 °C. The coverslip was removed gently after reaching room temperature. b) HLECs were embedded in 75 µl of a mixture of 50 µl cell suspension and 50 µl of 2% low melting point agarose (LMPA) at 37 °C, covered with a coverslip and refrigerated for 10 min at 4 °C. The coverslip was removed gently after reaching room temperature. A final layer of 1% HMPA was applied similarly. c) Cell lysis was performed by immersing the slides in ice lysis solution at 4 °C for 8 hours. Slides were then placed in a tank with denaturing buffer (NaOH 0.3 M, EDTA, Na2 1.0 mM) for 30 minutes, ensuring that the buffer level was 3-4 mm above the gel. Electrophoresis was conducted for 30 minutes at 25 volts. The slides were neutralized for 5 minutes and air-dried. Then, 100 microliters of ethidium bromide was added and covered with a coverslip. The slides were incubated in the dark for 30-40 minutes and washed with water. After drying, they were examined under a fluorescent microscope for an hour. At least 50 cells per sample were analyzed for DNA content in the nucleus and the tail by fluorescence intensity. The tail represents DNA damage caused by double-strand and single-strand breaks. The tail length and content are proportional to the DNA damage level measured by fluorescence intensity. Comet Score™ software, also known as Comet score, was used to estimate the ratio of DNA content in the head and tail of the comets, which reflects the amount of normal and damaged DNA respectively.

Lipid peroxidation was measured by MDA assay. Plasma (0.2 ml) was mixed with TBA-TCA-HCl reagent (0.8 ml) and heated in a water-bath for 15 min. The supernatant was centrifuged and its absorbance was read at 535 nm. The MDA level was calculated using an extinction coefficient of 1.56 × 105 /M/cm. LEC were homogenized in water and centrifuged at 12,000 g at 4°C for 30 min. The homogenate was used as the sample for MDA assay instead of plasma.

Statistical analysis:

GraphPad Prism 9 (United States) was used to compare the means of the two groups, an unpaired sample t-test was used: This indicates that the statistical comparison between the means of two independent

groups was conducted using a t-test. The t-test is a commonly used statistical test to determine if there is a significant difference between the means of two groups. 2. For two group simple linear regression, a p-value of 0.05 or less was considered statistically significant: This suggests that a simple linear regression analysis was conducted on data from two groups. In this analysis, a p-value of 0.05 or less was used as the threshold for determining whether the relationship between the two variables was statistically significant. A p-value less than 0.05 suggests that the relationship is unlikely to be due to chance.

Results:

The study included 30 rhexis specimens obtained from patients with senile cataract, who had a mean age of 64.6 ± 8.24 (Mean ±SD) years for males (n=15) and 63.6 ± 10.58 (Mean + SD) years for females (n=15). Additionally, 12 samples from cadavers were collected, with a mean age of 44.66 ± 4.7 (Mean ±SD) years for males (n=8) and 41.65± 4.02 (Mean + SD) years for females (n=4).

The comets assay revealed a significant increase in DNA damage in the cataract patients compared to the control subjects. The mean percentage of DNA in the tail of the comets was 36.01 ±9.59 for the cataract group and 27.31 ± 6.01 for the control group (Table 1). The difference was highly significant ($p<0.001$).

Table 1 . Results of Comet assay of HLEC in Senile Cataract and Control

Percentage of DNA DAMGE in HLEC in Senile Cataract	Percentage of DNA DAMGE in HLEC in Control	P Value
36.01 ±9.59	27.31 ± 6.01	p<0.001

The value of malondialdehyde, a marker of oxidative stress, was measured in both groups. The mean value of malondialdehyde was 0.3567 ± 0.1358 nmol/mg protein for the control subjects and 1.31 ± 0.38 nmol/mg protein for the cataract patients (Table 2). The difference between the two groups was statistically significant ($p<0.001$), indicating higher oxidative stress in cataract patients.

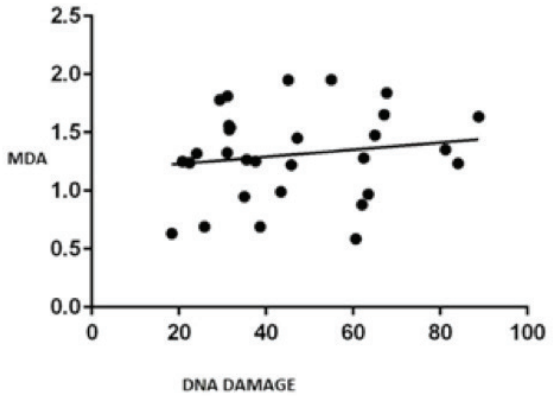
Table 2 . Results of malondialdehyde of HLEC in Senile Cataract and Control

Percentage of DNA DAMGE in HLEC in Senile Cataract	Percentage of DNA DAMGE in HLEC in Control	P Value
1.31 ± 0.38 nmol/mg protein	0.3567 ± 0.1358 nmol/mg protein	p<0.001

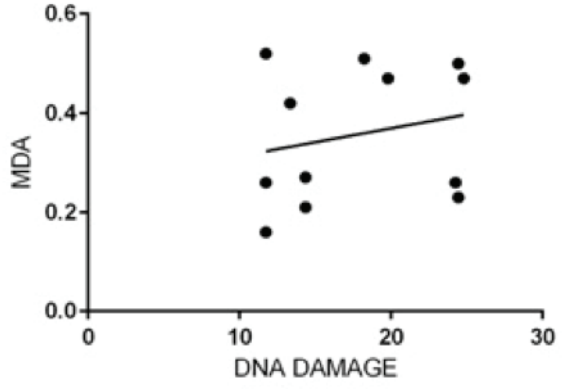
As shown in Figure 1, the levels of oxidative DNA damage and malondialdehyde (MDA), a marker of lipid peroxidation, were not significantly correlated in the senile cataract group.

As shown in Figure 1, the levels of oxi-

dative DNA damage and malondialdehyde (MDA), a marker of lipid peroxidation, were not significantly correlated in the senile cataract group.



Similarly, Figure 2 demonstrates that there was no significant correlation between these two parameters in the control group.



Discussion

Oxidative mechanisms are widely accepted as a major factor in the pathogenesis of cataract, particularly age-related cataract. [13] In vitro experiments have demonstrated that oxidative stress can induce cataract formation (Spector et al. 1993). In various forms of cataracts, including age-related cataract, proteins, lipids and DNA are extensively damaged by oxidative stress . [13-15] Oxidative stress has also been associated with elevated levels of lipid peroxidation products such as MDA in cataract patients (Micelli-Ferrari et al. [16] Hashim & Zarina [17] . In the present study, the higher MDA concentrations observed in the HLEC of cataract patients are consistent with the results of previous studies Micelli-Ferrari et al. [16] & Bhatia et al. [18] .This study investigated the possibility of DNA damage resulting from oxidative damage-induced lipid peroxidation. Oxidative stress and lipid peroxidation induced by inflammation, excess metal storage and excess caloric intake cause generalized DNA damage, producing genotoxic and mutagenic effects. The measurement of DNA damage shows the level of oxidative damage clearly because DNA damage is repaired well by cellular enzymes unlike the oxidation damage to other biomolecules that are not repaired and/or have a slow turnover such as lipids or proteins. [9]

A recent study by Goyal M M et al.[19] investigated the oxidative DNA damage in cataract patients by measuring the 8-OHdG levels in their leukocyte DNA. The study also measured the plasma MDA levels, which are a marker of lipid peroxidation, to assess the oxidative damage to

lipids. The findings revealed that cataract patients had significantly higher levels of both leukocyte 8-OHdG and plasma MDA than healthy controls, indicating enhanced oxidative stress and DNA damage. Furthermore, a significant positive correlation was found between leukocyte 8-OHdG and plasma MDA levels, implying a relationship between lipid peroxidation and DNA damage in cataract patients. Pan et al., [20] conducted a study to examine the levels of oxidative stress in type 2 diabetes patients with or without eye complications. They measured the amounts of MDA and 8-OHdG, which are markers of oxidative damage to lipids, proteins and DNA, respectively. They found that type 2 diabetes patients had significantly higher levels of MDA and 8-OHdG than healthy controls, and that those with eye problems had even higher levels than those without. This suggests that oxidative stress is involved in the development of both diabetes and its eye complications. [21]

We measured the amount of DNA damage and MDA, a marker of oxidative stress, in the cells that cover the lens of people with cataracts and compared them to a healthy group. We observed a higher level of MDA and DNA damage in the cataract group than in the control group. However, there was no statistically significant relationship between the DNA damage and the MDA levels in the lens cells of the cataract patients. Studies have shown that increased levels of malondialdehyde (MDA) are present in the lens and aqueous humor of cataract patients compared to healthy individuals. MDA has the capability to interact with nucleic acids, specifically DNA, which can result in multiple types of DNA damage, such as the creation of DNA adducts, breaks in the DNA strands, and the formation of DNA cross-links. This finding suggests that , DNA damage can also result from other oxidative factors.

This research supports the idea that oxidative stress may be a contributing factor to the development of cataracts, which aligns with previous studies. However, additional research is necessary to comprehensively comprehend the impact of oxidant stress on cataract formation. Future investigations in this field will assist in providing more substantial evidence and a more concise comprehension of the precise mechanism underlying the development of cataracts.

Conclusion

In conclusion, this research suggests that oxidative stress plays a role in the development of cataracts. Although it aligns with previous studies, further research is needed to fully understand the impact of oxidant stress on cataract formation. Fu-

ture investigations will help provide more robust evidence and a better understanding of the specific mechanism underlying cataract development.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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