ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 16, Issue 3, 2023

Online - 2455-3891
Print - 0974-2441

Research Article

DNA DAMAGE IN LENS EPITHELIAL CELLS OF SENILE CATARACT PATIENTS OF DIFFERENT PRAKRITI ACCORDING TO AYURVEDA LITERATURE

AVINASH NAMDEO JADHAO[1]*, KRANTI SANTOSH SORTE GAWALI[1], MANOJ CHANDRAKANT LOKHANDE[2], PARATE SHRAVANI S[3]

[1]Department of Biochemistry, Seth GSMC and KEMH Parel, Mumbai, Maharashtra, India. [2]Department of Biochemistry, Lokmanya Tilak Municipal Medical College and General Hospital Sion, Mumbai, Maharashtra, India. [3]Department of Kayachikitsa, Ayurved Seva Sangh, Nashik, Maharashtra, India. Email: avinashjadhao@kem.edu

Received: 31 October 2022, Revised and Accepted: 08 December 2022

ABSTRACT

Objectives: The Ayurvedic concept of the constitution is useful in predicting an individual's susceptibility to age-related diseases like Cataracts (Kaphaja Linganasha). The objectives of the study were to assess DNA damage directly in human lens epithelial cells (HLEC) of senile cataracts of *Vata* Predominant, *Pitta* Predominant, and *Kapha* Predominant *Prakriti* individuals.

Methods: After obtaining Institutional Ethics Committee permission, HLEC were taken from 20 *Vatta* Predominant,20 *Pitta* Predominant and 20 *Kapha* Predominant *Prakriti* individuals of cataract after cataract surgery and from 4 controls in which quantitative assessment of DNA damage were measured using CometScore™ software. The formation of "comets" in the DNA of lens epithelial cells can be visualized through the method of single gel electrophoresis and indicates DNA strand breaks, as the damaged DNA migrates at a different rate than non-damaged DNA during electrophoresis.

Results: No such prominent comets were indicating any DNA damage in the HLEC of the four control subjects, but comets were found in cataractous HLEC. The maximal damage was found in *pitta-predominant Prakriti* Individuals. In senile cataract patients, in HLECs DNA was randomly damaged and this type of damage was possible by reactive oxygen species. The DNA damage in HLEC was found maximally in *pitta* Predominant *Prakriti* individuals of senile type of cataract patients. Statistical significance was observed between senile cataracts in *pitta* predominant Prakriti versus senile cataracts in *Vata* predominant Prakriti individuals and between senile cataracts in *Vata* predominant Prakriti versus senile cataracts in *Kapha* Prakriti individual. No statistically significant results were obtained for senile cataracts in *pitta Prakriti* versus senile cataracts in *Kapha Prakriti* individuals.

Conclusion: The pathogenesis of senile cataracts is multifactorial and includes continuous molecular stress brought by photo-oxidative stress, UV irradiation, and oxidative reactions.

Keywords: Cataract, PRAKRITI, Comet assay.

 DOI: http://dx.doi.org/10.22159/ajpcr.2023v16i3.46712. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Cataract development accounts for 50% of blindness in the world [1]. A cataract is a senile change that happens because of aging. As age advances, cataract affects vision and if left uncorrected causes partial or complete blindness, this trouble seems the main concern of the twenty-first century [2]. The human lens epithelial cells (HLEC) perform a primary role in metabolic activities in lenses. Oxidative DNA damage to HLEC has long been documented as an important mediator in caspase-mediated cell death and has a major role in cataract pathogenesis [3,4]. Exposure of eyes to ultraviolet radiation causes photo-oxidative stress to generate reactive oxygen species such as superoxide radical anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (diatomic molecule $^{\bullet}$OH.), and singlet oxygen ($^1O_2$) attack cellular components, which is liable for DNA damage [5-8]. Various studies confirmed the association between a variety of DNA-damaging insults to the HLEC and the progress of lens opaqueness. The Ayurvedic concept of the constitution is useful in predicting an individual's susceptibility to a particular disease such as VattaPrakriti's individual predisposition for neurological disorders, dementia, movement and speech disorders, and arrhythmias, Pitta Prakriti individuals have a predisposition for skin diseases and ulcers, while Kapha predominant individuals have a predisposition for obesity, heart diseases and Type 2 diabetes mellitus. No other previous studies have directly measured *in vitro* assessment of DNA damage in lens epithelial cells of senile cataracts (KaphajaLinganasha) [9] of *Vata* Predominant, *Pitta* Predominant, and *Kapha* Predominant *Prakriti* individuals.

Our study was conducted to assess the damage of DNA in senile cataracts directly in HLEC based on *Prakriti* of Individuals.

METHODS

This work has been sanctioned by the "Institutional Ethics Committee" vide letter No. DMIMSU/IEC/2008-09/151dt May 30, 2008.

Study population

Sixty individuals from three predominant *Prakriti*, each consisting of 20 *Vata* Pridominant, 20 *Pitta* Predominant and 20 *Kapha* Predominant *Prakriti* patients compared among each other between age groups 60 and 80 years. As it is difficult to get healthy control, who can donate lenses. For running control, lenses of 4 dead individuals below age 40 were collected from our institute's mortuary who died in a road traffic accident. We have removed lenses from an expert but the procedure is not the same as cataract surgery.

Exclusion criteria

Type II Diabetes mellitus, high blood pressure, tobacco chewers, cigarette smokers and alcoholics, or any other major illness.

Prakriti assessment

Prakriti assessment was carried out by a questionnaire developed by (Tripathi and Gehlot 2019) [10]. Confirmation of all the characteristics done by an Ayurvedic physician (Fourth author) based on a meeting and a complete physical inspection to assess various physical, physiological,

and psychological characteristics as described in the questionnaire. The individual was classified into Vata *predominant*, Kapha *predominant*, and pitta *predominant*.

Detailed examination of senile cataract patients

The participants obtained a detailed ocular history. Pre-operative confirmation of cataracts and their type was recorded and confirmed by Professors of the Ophthalmology Department of Our Institute.

The procedure followed for removing the lens anterior capsule from senile cataract patients. Cataract patients were operated under local anesthesia using an injection of 2 mL 2% lignocaine, through a clear corneal incision (2.75 mm in length was made using a 2.2-mm double-blade corneal knife), 5.5 mm continuous curvilinear capsulorhexis was done by with the help of capsulorhexis forceps and 25-gauge needle. The anterior capsule was extracted in all cases with Visco expression through a clear corneal incision and the anterior capsule was collected using an experienced surgeon's forceps. No further handling or irrigation was done to avoid any direct harm to the HLEC. Once the removal anterior capsule is removed, the sample was instantly kept in an Essential Medium (containing 10% fetal bovine serum) and immediately transferred to the Research laboratory. A single rhexis for preservation was kept in Minimal Essential Medium, containing 10% fetal bovine serum, and incubated in an incubator containing 5% carbon dioxide at 37°C [11]. The maximum time-lapse from the collection of the sample to the process was 15–20 min. HLEC viability testing: Before starting the comet assay, the Trypan blue exclusion test was used to check if the HLEC were viable or not [12]. All collected samples were viable.

Sample preparation

Mechanical shaking technique was used for the preparation of cell suspension of the HLEC using the capsule (in 50 µl of Dulbecco's phosphate-buffered saline with pH 7.2), by hand, for a duration of up to 15 min at 4°C; HLEC were shaded from the lens capsule. To study the degree of DNA damage in HLEC of senile cataract patients, cell suspension of the lens epithelial cells was used for Comet assay**.** For conducting the Comet assay, we followed the steps which were designed by Singh *et al*. [13], with some variations: (1) Precoating of glass microscopic slides: 75 µL of 1% of high melting point agarose at 65°C was dropped onto the slide, covered with 18 mm × 18 mm coverslip and was kept at 4°C for 10 min. Then the slide was kept outside to attain room temperature and carefully removed coverslip so that the gel is not damaged. (2) Embedding of HLEC: Mix 50 µL of cellular suspension(quantification of the cell was not done, the approximate sample was taken**)** in 50 µL with 2% low melting point agarose (LMPA) at 37°C, then 75 µL of this mixture was dropped onto the slide, covered with a coverslip, and kept at 4°C for 10 min. Then, the slide was kept outside to attain room temperature, and carefully removed coverslip. Then, the last layer of 1% LMPA was applied on the slide in the same manner; and (3) lysis of lens epithelial cells was done by dipping the slide in the ice lysis solution (NaCl 2.5 M, EDTA, Na_2 100 mM, Tris 10 mM) at 4°C for 8 h instead of 2 h. Slides were kept in a tank containing a denaturing buffer (NaOH 0.3 M, EDTA, Na_2 1.0 mM) for 30 min. The level of the solution is to be kept 3–4 mm above the gel and the process of electrophoresis was carried out for a duration of 30 min, by applying an electric current of 25 volts. Slides were washed with a neutralizing solution (Tris HCl, o.4M, pH7.5) for 5 min and were kept at room temperature for drying. It takes 30 min. For visualization, drop 100 ml of ethidium bromide solution (10 mg/ml in water) onto the slide and it is covered by a coverslip. Incubated for 30–40 min in the dark and then rinsed with water. It was dried (no air drying) and observed under a fluorescent microscope within 1 hour. No less than fifty cells from each sample were counted and the amounts of DNA in the main body and tail were measured by fluorescence intensity. Samples are handled separately based according to Ayurvedic classification. The amount of damage is represented by an increased fragment of DNA in the nucleus similar to the tail of a comet. The DNA fragments are generated by the break of DNA double-strand and single strand; the length and fragment content of the tail is directly proportional to the extent of DNA damage measured by fluorescence intensity. Photographs were taken for analysis as shown in Fig. 1. The ratio of DNA content in the head and tail (normal DNA versus damaged DNA) was estimated by CometScore™ software called Cometscore.

Statistical analysis

Mean values and standard deviation had been used to define data in 20 *Vata* Predominant, 20 *Pitta* Predominant, and 20 *Kapha* Predominant *Prakriti groups.* Student unpaired "t" test was used to test the significance between the above three groups. SPSS ver. 15.0 was used for all statistical analyses.

RESULTS

No projecting comets were observed in the HLEC of the control samples, but prominent comets were found in senile cataract participants of HELCs. The percentage of DNA damage in HLEC of Cadavers (otherwise healthy and died by road traffic accidents) the highest percentage of DNA damage in the tail was 20.89 % and the lowest was 7.89% (Table 1).

Patients with *Vata* predominant *Prakriti* with *the* highest DNA in the tail were found to be 51.88% and the lowest was 24.09% as shown in (Table 2).

The highest percentage of DNA damage in HLEC of senile cataracts in patients of *Pitta* predominant *Prakriti* was found to be 37.89% and the lowest was 21.44% as shown in (Table 3).

The highest percentage of DNA damage in HLEC of senile cataracts in patients of *Kapha* Predominant *Prakriti* was found to be 52.01% and the lowest was 21.55% as shown in (Table 4).

Statistical significance was observed between senile cataract in *VataPrakriti* versus senile Cataract in *PittaPrakriti* individual and Senile Cataract in *PittaPrakriti* versus Senile cataract in *KaphaPrakriti* individual. No statistically significant results were obtained from senile cataracts in *VataPrakriti* versus the Senile cataract in *Kapha Prakriti* individual as shown in (Table 5).

DISCUSSION

The lens epithelial cells are the major site of all cellular metabolic activities, the detoxification process of major free radicals, and the

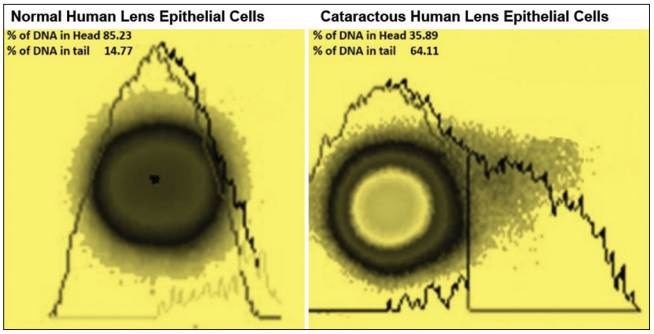

Fig. 1: Percentage of DNA damage in human lens epithelial cell

Table 1: Percentage of DNA damage in lens epithelial cells of cadavers (otherwise healthy, died by road traffic accidents)

Sample number (n=4)	Head (% DNA)	Tail (% DNA)
1	87.11	12.89
2	89.13	10.87
3	92.11	7.89
4	79.11	20.89
Mean	86.865	13.135
Standard deviation	5.563	5.563

Maximum DNA in the tail was found to be 20.89% and the minimum was 7.89%.

Table 2: Percentage of DNA damage in lens epithelial cells in senile cataract patients of *pitta* predominant *Prakriti* (n=20)

Sample number	Head (% DNA)	Tail (% DNA)
1	75.91	24.09
2	74.21	25.79
3	56.44	43.56
4	52.22	47.78
5	68.2	31.8
6	54	46
7	67.12	32.88
8	64.23	35.77
9	61.45	38.55
10	48.12	51.88
11	63.11	36.89
12	68.11	31.89
13	66.76	33.24
14	71.66	28.34
15	52.55	47.45
16	61.98	38.02
17	64.11	35.89
18	59.89	40.11
19	67.11	32.89
20	58.11	41.89
Mean	62.7645	37.2355
Standard deviation	7.532449	7.532449

Maximum DNA in the tail was found to be 51.88% and the minimum was 24.09%

Table 3: Percentage of DNA damage in lens epithelial cells in senile cataract patients of *Vata* predominant *Prakriti* (n=20)

Sample number	Head (% DNA)	Tail (% DNA)
1	71.28	28.72
2	68.91	31.09
3	68.88	31.12
4	68.44	31.56
5	68.32	31.68
6	65.11	34.89
7	62.11	37.89
8	65.36	34.64
9	68.12	31.88
10	69.12	30.88
11	78.56	21.44
12	62.56	37.44
13	70.88	29.12
14	65.33	34.67
15	68.99	31.01
16	65.78	34.22
17	76.55	23.45
18	73.55	26.45
19	69.22	30.78
20	69.65	30.35
Mean	68.836	31.164
Standard deviation	4.107224	4.107224

Maximum DNA in the tail was found to be 37.89% and the minimum was 21.44%

transport of water, glucose, and ions into and out of the lens moreover maximum mitochondrial oxygen consumption and generation of ATP through oxidative phosphorylation occur in the cells of the lens [14-18]. The pathogenesis of cataracts is multifactorial, with advancing age, ultraviolet radiation exposure, tobacco consumption, elevated sugar levels, and various photo-oxidative reactions [19,20]. We here examined HLEC of cataracts by Comet Assay and present the first report on the relative magnitude of the severity of DNA damages in all three types of cortical, nuclear, and sub capsular forms of cataracts. The purpose of our study was to examine the DNA damage in the HLEC of senile cataracts in *Vata* predominant, *pitta* predominant and *Kapha* predominant *Prakriti* individuals.

Table 4: Percentage of DNA damage in lens epithelial cells in senile cataract patients of *Kapha* predominant *Prakriti* (n=20)

Sample number	Head (% DNA)	Tail (% DNA)
1	57.18	42.82
2	50.67	49.33
3	59.1	40.9
4	68.88	31.12
5	56.77	43.23
6	68.32	31.68
7	66.62	33.38
8	66.31	33.69
9	65.36	34.64
10	65.23	34.77
11	68.86	31.14
12	61.11	38.89
13	75.33	24.67
14	65.12	34.88
15	47.99	52.01
16	60.98	39.02
17	72.11	27.89
18	54.97	45.03
19	71.92	28.08
20	78.45	21.55
Mean	64.064	35.936
Standard Deviation	7.759895	7.759895

Maximum DNA in the tail was found to be 52.01% and the minimum was 21.55%

Table 5: Statistical Analysis of DNA damage directly in lens epithelial cells of senile cataract patients of different *Prakriti*

Study group	"t" value	p-value	Significance
Senile Catract in *pitaprakriti* versus Senile Cataract in *vata prakriti*	3.165	0.0031	Significant
Senile Cataract in *vataprakriti* versus Senile Catract in *kaphaprakriti*	2.382	0.0223	Significant
Senile Cataract in *pitaprakriti* versus Senile Cataract in *kapha prakriti*	0.5302	0.599	Not Significant

In our study, we observed DNA damage in HLEC of Senile cataract individuals of different *prakriti's*. Therefore, in senile cataract patients, a maximum percentage of DNA damage was observed in individuals of *pitta-predominant Prakriti* individual. Both *Vata* and *Pitta* are responsible for degenerative changes due to their specific properties. Aging is the procedure of degeneration and thus intensified by the predominant *Pitta* supported by *Vata* [21]. HLEC DNA was haphazardly damaged, such harm is possible by free radicals generated by photo-oxidative reactions during cellular metabolism. The limitation of our study is as our sample size is small and also difficult to get healthy human lenses.

CONCLUSION

Maximum damage was found in individuals of *pitta* predominant *Prakriti* individuals. DNA damage is aggravated in *pitta-predominant* individuals. Oxidative stress and reduced activity of free radical scavenging enzymes may responsible for enhanced DNA damage in *pitta-predominant* individuals of senile cataract patients. Diet rich in Vitamins A, C, E, selenium, and various other antioxidants may delay the onset of cataracts.

ACKNOWLEDGMENT

Nil.

AUTHORS CONTRIBUTION

Concept writing: Avinash Namdeo Jadhao and Kranti Santosh Sorte Gawali, Manuscript writing, and Proofreading: Manoj Chandrakant Lokhande Grading of individual according to Ayurveda literature: Parate, Shravani S.

CONFLICTS OF INTEREST

Nil.

AUTHORS FUNDING

Nil.

REFERENCES

1. Pascolini D, Mariotti SP, Pokharel GP, Pararajasegaram R, Etya'ale D, Négrel AD, *et al*. 2002 Global update of available data on visual impairment: A compilation of population-based prevalence studies. Ophthal Epidemiol 2004;11:67-115. doi: 10.1076/opep.11.2.67.28158, PMID 15255026
2. Brian G, Taylor H. Cataract blindness-challenges for the 21st century. Bull World Health Organ 2001;79:249-56. PMID 11285671
3. Spector A. Oxidative stress induced cataract: Mechanism of action. FASEB J 1995;9:1173-82. doi: 10.1096/fasebj.9.12.7672510, PMID 7672510
4. Wang X, Simpkins JW, Dykens JA, Cammarata PR. Oxidative damage to human lens epithelial cells in culture: Estrogen protection of mitochondrial potential, ATP and cell viability. Invest Ophthalmol Vis Sci 2003;44:2067-75. doi: 10.1167/iovs.02-0841, PMID 12714645
5. Ortwerth BJ, Coots A, James HL, Linetsky M. UVA irradiation of human lens proteins produces residual oxidation of ascorbic acid even in the presence of high levels of glutathione. Arch Biochem Biophys 1998;351:189-96. doi: 10.1006/abbi.1997.0549, PMID 9515056
6. Jose JG, Yielding KL. "Unscheduled" DNA synthesis in lens epithelium following ultraviolet radiation. Exp Eye Res 1977;24:113-9. doi: 10.1016/0014-4835(77)90252-4, PMID 851489
7. Kojima M. Regional enzymatic analysis of UV-B and streptozotocin induced diabetic cataract lens. Lens Eye Toxic Res 1990;7:547-61. PMID 1966039
8. Kuck JF Jr. Effect of long wave ultraviolet light on the lens. IV. Leucine metabolism in normal human lenses *in vitro* Ophthalmic Res 1983;15:216-9. doi: 10.1159/000265262, PMID 6634057
9. Dhiman KS, Dhiman K, Puri S, Ahuja D. A comprehensive review of cataract (Kaphaja Linganasha) and its surgical treatment in ayurvedic literature. Ayu 2010;31:93-100. doi: 10.4103/0974-8520.68197, PMID 22131692, PMCID PMC3215330
10. Tripathi PK, Gehlot S. Development, validation and confirmation of an archetype tool to evaluate Prakriti. J Nat Rem 2019;19:206-13. doi:10.18311/jnr/2019/23788
11. Gajjar D, Patel D, Alapure B, Praveen MR, Patel A, Johar K, *et al*. Rapid action of oestradiol against hydrogen peroxide induced oxidative stress in cataractous lens epithelium: An *in vitro* study. Eye (Lond) 2008;23:1456-63.
12. Nanavaty MA, Johar K, Sivasankaran MA, Vasavada AR, Praveen MR, Zetterström C. Effect of trypan blue staining on the density and viability of lens epithelial cells in white cataract. J Cataract Refract Surg 2006;32:1483-8. doi: 10.1016/j.jcrs.2006.04.017, PMID 16931259
13. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988;175:184-91. doi: 10.1016/0014-4827(88)90265-0, PMID 3345800
14. Bhat SP. The ocular lens epithelium. Biosci Rep 2001;21:537-63. doi: 10.1023/a:1017952128502, PMID 11900326
15. Bassnett S, McNulty R. The effect of elevated intraocular oxygen on organelle degradation in the embryonic chicken lens. J Exp Biol 2003;206:4353-61. doi: 10.1242/jeb.00670, PMID 14581604
16. Eaton JW. Is the lens canned? Free Radic Biol Med 1991;11:207-13. doi: 10.1016/0891-5849(91)90173-z, PMID 1937139
17. Holekamp NM, Shui YB, Beebe DC. Vitrectomy surgery increases oxygen exposure to the lens: A possible mechanism for nuclear cataract formation. Am J Ophthalmol 2005;139:302-10. doi: 10.1016/j.ajo.2004.09.046, PMID 15733992
18. Shui YB, Fu JJ, Garcia C, Dattilo LK, Rajagopal R, McMillan S, *et al*. Oxygen distribution in the rabbit eye and oxygen consumption by the lens. Invest Ophthalmol Vis Sci 2006;47:1571-80. doi: 10.1167/iovs.05-1475, PMID 16565394
19. Vinson JA. Oxidative stress in cataracts. Pathophysiology 2006;13:151-62. doi: 10.1016/j.pathophys.2006.05.006, PMID 16765571
20. Tang Y, Wang X, Wang J, Jin L, Huang W, Luo Y, *et al*. Risk factors of age-related cataract in a Chinese adult population: The Taizhou eye study. Clin Exp Ophthalmol 2018;46:371-9. doi: 10.1111/ceo.13040, PMID 28842942
21. Purvya MC, Meena MS. A review on role of Prakriti in aging. Ayu 2011;32:20-4. doi: 10.4103/0974-8520.85719, PMID 22131753