Study of Genotoxic Effects of Artificially Induced UV-Radiation on Human Lens Epithelial Cell (HLEC) Culture by Using Dye Exclusion Assay

Huddar MD, Paikrao VM*

Department of Anatomy, NKP Salve Institute of Medical Sciences and Research Centre and Lata Mangeshkar Hospital, Digdoh Hills, Hingna Road, Nagpur

ABSTRACT

Cataract is one of major cause of blindness, 44% cases of blindness is due to cataract only. In developing countries such as India cataract is more common, and it manifest earlier in life than the most developed countries of the world. Cataract is more common in dry and hot area where sun (UV) exposure is more than prolong cloudy cover. In this study we have aimed to compare effects of UV-ray irradiation on cell viability of HLEC (Human lens epithelial cell) culture by using trypan blue dye exclusion assay against the unexposed control HLEC culture. Total 20 cataract patients from Dept. of Ophthalmology of Lata Mangeshkar Hospital, Nagpur were included in this study. The capsulorhexis sample from cataract surgery taken for study from eye OT. The capsulorhexis sample without any UV-ray exposure taken as control. For culture of HLEC’s RPMI 1640 medium containing 10% fetal calf serum used. The HLEC cultures were UV-ray irradiated with increasing time exposure PHILIPS® TUV 15W/G15 T8 ultraviolet UV rays tube. The Trypan blue dye exclusion assay was used to analyze cell viability. The slides were analyzed using Olympus® BX51 Research microscope with oil immersion and 100 were observed per sample. The cell viability was calculated using Epi info® (version 6.0), as subject means with standard deviation. Statistical significance will be defined at P≤0.001. Out of 100 cases the 12 were female and 8 were male. Mean age of both male and female patients were 62.45±3.18(SD). A positive linear correlation was found (r=0.819, p<0.001) between the time of UV-Ray exposure and death cells per 100 observed LECs.

Key words: Human lens epithelial cells, UV rays, Trypan blue.

INTRODUCTION

Cataract is one of the major causes of blindness. 44% cases of blindness are due to cataract only. In developing countries such as India cataract is more common, and it manifests earlier in life than the most developed countries of the world [1]. Years of exposure to UV rays cause the protein in the lens of the eye to clump and thicken, preventing light from passing through it. This clouding and thickening of the lens is known as a cataract, and it is the leading cause of vision loss in the world. The cataract caused by ionising radiation as UV-ray, different from that caused by age [2]. The age-related cataracts are most commonly found in the nuclear region and cortical cataracts are commonly found in diabetic patients while the ionising radiation is generally although not exclusively associated with posterior sub capsular and sometimes cortical opacities [3]. UV rays can damage our vision every day, deteriorating the lenses of our eyes. Too much time in the sun can speed cataract development, so it is important to limit your time in direct sunlight. Not wearing proper eye protection is another culprit, so keep in mind that sunglasses are equally important in the winter as well as the summer. Daily choices that you make can protect your precious eyesight. Hats, wraparound sunglasses, and lenses that offer 100 percent UVA and UVB protection can help prevent, or at least delay, cataract formation.

Giblin et al. [4] previously reported on how long-term exposure to low-dose UVA induced the development of nuclear cataract (NUC) in a guinea pig model [5]. Although many previous epidemiological studies have reported an association between cortical cataract (COR) and UV light [6-8], most of these studies were
conducted in regions ranging from mid- to high-
latitudes. To date, only a few studies have been
conducted in low latitude regions with strong UV
exposure. Some studies investigating NUC have
reported no association with UV exposure [5,8],
in contrast, others have reported relationships
between these factors [9-13]. In addition, some
reports have indicated an association between
UV exposure and posterior subcapsular cataract
(PSC) [14]. Retrodots (RD) and waterclefts (WC)
are cataract subtypes that cause decreased visual
function, with a high prevalence among middle-
aged to elderly individuals [15,16]. Because no
studies have investigated associations between
UV exposure and these types of cataract, their relationship with UV exposure remains
unknown. Moreover, few reports have examined
this relationship in a single race living in regions
with different UV intensities.

There have been few reports of the effects
of diagnostic UV-Ray exposure on the lens,
although number of researchers have suggested
that repeated exposure to UV-ray, computerized
axial tomography (CAT) scans radiation, drugs
reaction may have cataractogenic potential.

There is need to investigate the effects of UV-
ray irradiation on the eyes, though there is vast
literature on the effects of UV-ray irradiation on
body tissues and tissue cultured cells in vitro
and in vivo, these literature lack the work on
lens epithelial cells (LEC) of humans, such an
information would describe the actual relation
between the UV-ray irradiation and viability in
human lens epithelial cells (HLEC).

AIMS AND OBJECTIVES

In this study we aimed to comparing the effects
of UV-ray irradiation on HLEC Culture by
using trypan blue exclusion assay against the
unexposed control HLEC culture and give the
significant relation between UV-ray irradiation
on HLEC Culture and cell viability, if any.

MATERIALS AND METHODS

Patient selection: This study comprised
cataract patients of all ages, who had undergone
phacoemulsification. The informed consent was
obtained from the participants. The patients
lived in with the surroundings of contact of
radiation as UV-ray was excluded from study.

Sample collection: The circular pieces of human
anterior lens capsulorhexis sample was collected
post operatively in sterile normal saline from
cataract patients from the eye OT of institute's
hospital. The type of cataract was noted as per
lens opacities classification (LOC) system III [17].

Tissue Culture of Human Lens Epithelial Cell
(HLEC): Sterilisation of lab ware and culture
room. Cleaning and sterilisation of glassware
were carried out by the standard protocol
using appropriate disinfectants followed by
autoclaving of glassware at 15 lbs for 20mins.
Inoculation room was fumigated with 5%
formaldehyde spray in the culture room. The UV
light was switched on for half an hour, before
preparing the culture media. RPMI 1640 media
used to culture the HLECs.

Human lens epithelial cell (HLEC) culture:
Cell culture was carried out as per Goyal et al.
[18] to acclimatize the HLEC Cells, a single rhexis
was placed in 1ml of RPMI medium containing
10% foetal calf serum in a single well of a 24 well
plate and was incubated for 1 hour 30 minutes
on appropriate CO₂ pressure at 37°C.

UV-Ray irradiated human lens epithelial cell
(HLEC) culture: The culture plate with human
lens epithelial cell (HLEC) culture was UV-ray
irradiated using PHILIPS® TUV 15W/G15 T8
ultraviolet UV rays' tube. For irradiation, the
increasing exposure time of UV-ray was used.
The control was without any UV-ray irradiation.

Trypan blue exclusion assay: The trypan blue
exclusion assay was used to ensure cell viability.
In this test, a few drops of trypan blue were
added on a rhexis, placed on a glass slide and
then microscopically examined to determine
whether cells take up or exclude the dye. A viable
cell had a clear cytoplasm whereas a nonviable
cell had a blue cytoplasm [19].

Slide preparation: The rhexis was removed
from the trypan blue dye and was washed twice
in PBS (phosphate buffer solution). Then rhexis
was immediately mounted on a drop of PBS,
on clean dry grease free slide. The slides were
analysed using Olympus Research microscope
with oil immersion and 1,000 cells were
observed per sample. Only nucleated cells that
were separated without overlapping or folds
were analysed.
Statistical analysis: Statistical analysis was carried out using the Epi Info® statistical package, after breaking the code. The cell viability was calculated as subject means with the standard deviation. Statistical significance was defined at P ≤ 0.05.

RESULTS

Out of 20 cases the 12 were female and 8 were male. Mean age of both male and female patients were 62.45 ± 3.18(SD). A positive linear correlation was found (r: 0.819, p<0.001) (Graph 1) between the time of UV-ray exposure and death cells per 100 observed LECs (Figure 1).

Figure 2 shows the cell death. Results of UV-ray exposed LECs with UV-ray exposure are mentioned in Table 1 and results of controls LECs without any UV-ray exposure are mentioned in Table 2.

Figure 1: Trypan blue dye exclusion assay. (a) Controls LECs without any UV-ray exposure; (b) UV-ray exposed LECs.

![Figure 1](image1)

![Figure 2](image2)

Table 1: UV-ray exposed LECs with UV-ray exposure.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Time of UV-ray exposure (in min)</th>
<th>Death cells observed in Culture (per 100 cells Observed)</th>
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DISCUSSION

Cataract is the multifactorial disease. Various causative factors are behind the development of cataract. Though the age-related cataract is more prevalent the other factors also play an important role in the pathogenesis of cataract. Beside this cataract is more common in dry and hot area where sun (UV) exposure is more than prolong cloudy cover.

A lot of researchers worked on the pathogenesis of cataract but most of them were concentrated on the age-related cataract and some on non ionising radiation. Therefore, it was significant to know the comparative effects of UV-Ray irradiation on HLEC against the unexposed control HLEC Culture. From the proposed research we have achieved the viability of the HLEC in the UV-Ray radiation and determined the minimum time through UV-Ray exposure.

According to Robert, et al. [20] radiation exposure can lead to impaired vision and transient or permanent blindness. Both ultraviolet-A (UV-A) and UV-B induce cataract formation and are not necessary for sight. Ultraviolet radiation is also a risk factor for damage to the retinas of children. The removal of these wavelengths from ocular exposure will greatly reduce the risk of early cataract and retinal damage. One way this may be easily done is by wearing sunglasses that block wavelengths below 400 nm (marked 400 on the glasses). However, because of the geometry of the eye, these glasses must be wraparound sunglasses to prevent reflective UV radiation from reaching the eye. Additional protection may be offered by contact lenses that absorb significant amounts of UV radiation. In addition to UV radiation, short blue visible light (400-440 nm) is a risk factor for the adult human retina. This wavelength of light is not essential for sight and not necessary for a circadian rhythm response. For those over 50 years old, it would be of value to remove these wavelengths of light with specially designed sunglasses or contact lenses to reduce the risk of age-related macular degeneration.

According to American Optometric Association [21] Ultraviolet (UV) radiation comprises invisible high energy rays from the sun that lie just beyond the violet/blue end of the visible spectrum. More than 99% of UV radiation is absorbed by the anterior structures of the eye, although some of it does reach the light-sensitive retina. The UV radiation present in sunlight is not useful for vision. There are good scientific reasons to be concerned that UV absorption by the eye may contribute to age-related changes in the eye and several serious eye diseases. Protection can be achieved by simple, safe, and inexpensive methods such as wearing a brimmed hat and using eyewear that absorbs UV radiation.

Krivandin et al analyzed diffraction, structural conversions of crystallins in human lens were detected in senile cataract and upon artificial dehydration of lens tissue. In senile cataract certain characteristics of the native three-dimensional structure of γ- and β-crystallins are completely lost, whereas during dehydration of lens tissue a small but significant contraction of these protein molecules takes place. Upon artificial UV-irradiation of bovine crystallins destructive changes are observed, which are remarkably similar to those in cataract.

Apart from the scientific research we interacted with the patients, the knowledge of research designing and the exposure to the scientific protocols and biostatistics analysis.

We collect data regarding alcohol consumption of cataract patients of Vidarbha region, our data also support all research report. We also found risk of cataract more in alcohol drinker.

CONCLUSION

From this research it can be concluded that the prolong exposure of UV-ray dose might be lethal to the LECs and it may have cataractogenic potential, as these cells are precursor of eye lens.

CONFLICT OF INTEREST

The none to declare.
ACKNOWLEDGMENTS

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