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Original Article

MULTILAYERING OF CENTRAL ZONE LENS EPITHELIAL CELLS IN HUMAN CATARACTS

KAID JOHAR, S. R.[1], TATSUMI, K.,[2] AND VASAVADA, A.[1]

[1]Iladevi Cataract & IOL Research Centre, Ahmedabad, India; [2]Department of Anatomy II, Nara Medical University, Nara, Japan

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Abstract: ***The lenticular epithelium (LE) is arranged in monolayer at the anterior surface of the lens below lens capsule. Due to stress, the lens epithelial cells (LECs) extrude out of the monolayer, get superimposed and become multilayered. In the present study, we have detected the number of superimposed cells in different types of cataracts using flat preparations along with the study of semithin sections and immunofluorescence labeling of proliferative cells by labeling topoisomerase II. LE obtained from clear cadaver lenses served as a control. Superimposition of cells was not found in the capsulorhexis samples obtained from clear lenses and nuclear cataracts while it was highest in the posterior subcapsular cataract followed by cortical and mix type of cataracts. Semithin section studies indicate the relationship of the superimposed cells with the surrounding cells. The number of proliferative cells was highest in the posterior subcapsular cataract followed by cortical cataract, mix cataract, clear lenses and nuclear cataract. Superimposition of LECs is found in posterior subcapsular, cortical and mix types of cataracts and is associated with higher proliferation.***

Keywords: Multilayering of the lens epithelium, Human cataracts

Dr Kaid Johar SR is Associate Professor at Department of Zoology, School of Sciences, Gujarat University, Ahmedabad. He has expertise in cell and molecular biology and has experience in working with various ocular diseases, neurodegenerative diseases and cancer. The primary focus of his research is on understanding epithelial mesenchymal transition and integration of cellular activity with the metabolism of cells at the molecular level. He has published more than 60 papers in reputed international and national journals. He has presented his work at more than 30 international and national conferences.

Dr AbhayVasavada is the Founder and Director of Iladevi Cataract and IOL Research Centre, Ahmedabad. He is well known Ophthalmologist and is equally interested in perusing research. The primary focus of his research is understanding, evolving and educating the newer techniques of ocular surgeries. He is keen on applying the basic research techniques in understanding ocular pathophysiology. He has published more than 150 papers in various international and national journals. He is the author of several books and book chapters. He has received several international and national awards

Dedication: I have been associated with Dr P. D. Gupta for more than 25 years. Right from the days of my PhD to as a colleague at Iladevi Cataract and IOL Research Centre, Ahmedabad, Dr P. D. Gupta helped me understand various aspects of cell and molecular biology. I am feeling very honored to write this article on his 85thbirthday.

INTRODUCTION

The lenticular epithelium (LE) consists of monolayer of cuboidal epithelial cells underlying the lens capsule. Based on the proliferative kinetics and morphology the LE is divided into central (CZ), germinative and equatorial zones. The cells of the CZ are normally devoid of proliferative activity and are arrested in the G1 phase of the cell cycle [1,2]. Nevertheless, these cells do mitoses under stress conditions arising due to severe injury, influenced by hormones, exposed to inflammatory and toxic insults, etc [3-5]. The cells of the germinative zone are mitotically active and upon division move posterior to the equatorial zone where morphological and biochemical differentiation occur which leads to the formation of lens fibers. Nuclei and other cell organelles such as rough and smooth endoplasmic reticulum, mitochondria etc. are lost as the lens epithelial cells (LECs) differentiate to form the lens fibers [6]. The life maintaining processes of the avascular lens are thus restricted to the monolayer LECs [7].

Cellular alterations in human LECs have been shown in some cataract types [8]. Cellular division of the LECs of CZ have been shown in experimental animal models by various workers [9-23,26]. However, the superimposition or extrusion followed by localized multilayering of cells of the lens epithelium is not shown in various types of human cataract. In the present investigation, we have studied the superimposition of LECs in the CZ of different types of cataracts. To further confirm the relation of the extruded cells from monolayer with the surrounding cells, we have studied the superimposed cells in thin section microscopy. We have also determined the presence of proliferative cells by labeling of topoisomerase II using indirect fluorescence.

MATERIALS AND METHODS

Human eyeballs after the removal of corneal button were obtained from Indian Red Cross Eye Bank, Ahmedabad. The study was performed as per the declaration of Helsinki and was approved by Institutional Ethical Committee. Each eyeball was subjected to slit-lamp examination (SL 130, Zeiss, Germany) and only clear transparent lenses were considered as normal lenses. The eyeball was placed under the operating microscope (Zeiss, OPMI 1-FR, Germany) and the central zone of the lens epithelia was dissected after placing a small central nick and then by peeling off the anterior capsule in the form of continuous, circular capsulorrhexis of about 4-5 mm diameter. The anterior capsule with epithelium was processed further for different types of microscopic examinations.

Central zone of human cataract lens epithelia was obtained from patients undergoing cataract extraction. The cataracts were classified preoperatively into nuclear, cortical, posterior subcapsular, mature and mixed using slit lamp examination [23].

Light Microscopy: For flat mount preparations, the capsulorhexis samples from clear lenses and cataracts were spread on separate glass slides under the Olympus, Japan Stereozoom Microscope and fixed with Carnoy's fixative. These were stained with hematoxylin-eosin and observed under light microscope (Carl Zeiss, Germany) and photographed using digital camera (Sony, Japan). The number of superimposed cells were counted using Image Analysis software (Biovis, Expert Vision Lab, Mumbai, India).

The LE obtained from clear and cataracts LE were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) [24]. Following fixation in glutaraldehyde for three hours at room temperature the LECs were post fixed in 1% osmium tetroxide in 0.1M phosphate buffer (pH 7.2) for three hours. The osmium tetroxide fixed tissues were then washed, dehydrated through ascending grades of acetone and embedded in Araldite on flat embedding molds in the desired orientation. Semi-thick sections (0.5 µm)were cut on Reichert-Jung ultramicrotome fitted with a glass knife. Semi thick sections were collected onto clean glass slides, stained with 1% toludine blue and observed under light microscope.

Immunofluorescence: The capsulorrhexis specimens from 1 clear and 3 from each type of cataracts were obtained and immediately put on the glass slide in PBS and spread under dissecting microscope. Fixed with 1-4% paraformaldehyde in PBS, for 1 minute at room temperature. Again, washed with PBS, twice, for 5 minutes, specimen was covered with 1% BSA in PBS and incubated for 1 hour at room temperature. Washed with PBS for 15 minutes and the specimen was covered with 5 mg/ml anti-topoisomerase II in 1% BSA in PBS and incubated for 2-3 hours at room temperature. Washed twice with PBS for 5 minutes. Incubated with 1:200 dilution

of goat anti-rabbit IgG conjugated with AlexaFluor 488 in 1% BSA in PBS for 1 hour at room temperature. Washed with PBS for 15 minutes, counter stained with propidium iodide for 5 minutes and mounted with antifade reagent and examined under fluorescent microscope. Topoisomerase II positive cells were counted.

RESULTS

LE of the human clear lenses and posterior subcapsular, nuclear, cortical, mixed and mature types of human cataracts were studied by various microscopic techniques to examine change in the cell organization. The cells in the CZ of the clear lenses were arranged in a monolayer displaying characteristic cobblestone like arrangement. Superimposition of cells was observed in the posterior subcapsular, cortical and mixed type of cataracts (Fig. 1).

We have counted the number of superimposed cells in the central zone of lens epithelium of clear as well in cataracts. The number of superimposed cells were highest in the posterior subcapsular cataract followed by mixed and cortical cataracts. The number of superimposed cells in clear lenses and pure nuclear cataract samples was very less (Fig. 1).

Upon the observation of semithin sections stained with toludine blue under the light microscope, we were able to observe the superimposition of cells having their regular cell-cell interactions with surrounding cells. This observation rules out the possibility of released cells of LE to settle on the monolayer during processing (Fig. 2).

To detect the proliferative activity in the lens epithelial cells, we used topoisomerase II as a marker of cell proliferation and indirect immunofluorescence labeling with AlexaFluor 488-labeled second antibody. Propidium iodide was used as a counter stain. We further calculated the number of topoisomerase II labeled cells in the central zone lens epithelium obtained from clear lenses and cataracts. The

Fig. 1: Superimposition of cells (arrow) in human clear lens and different cataracts.

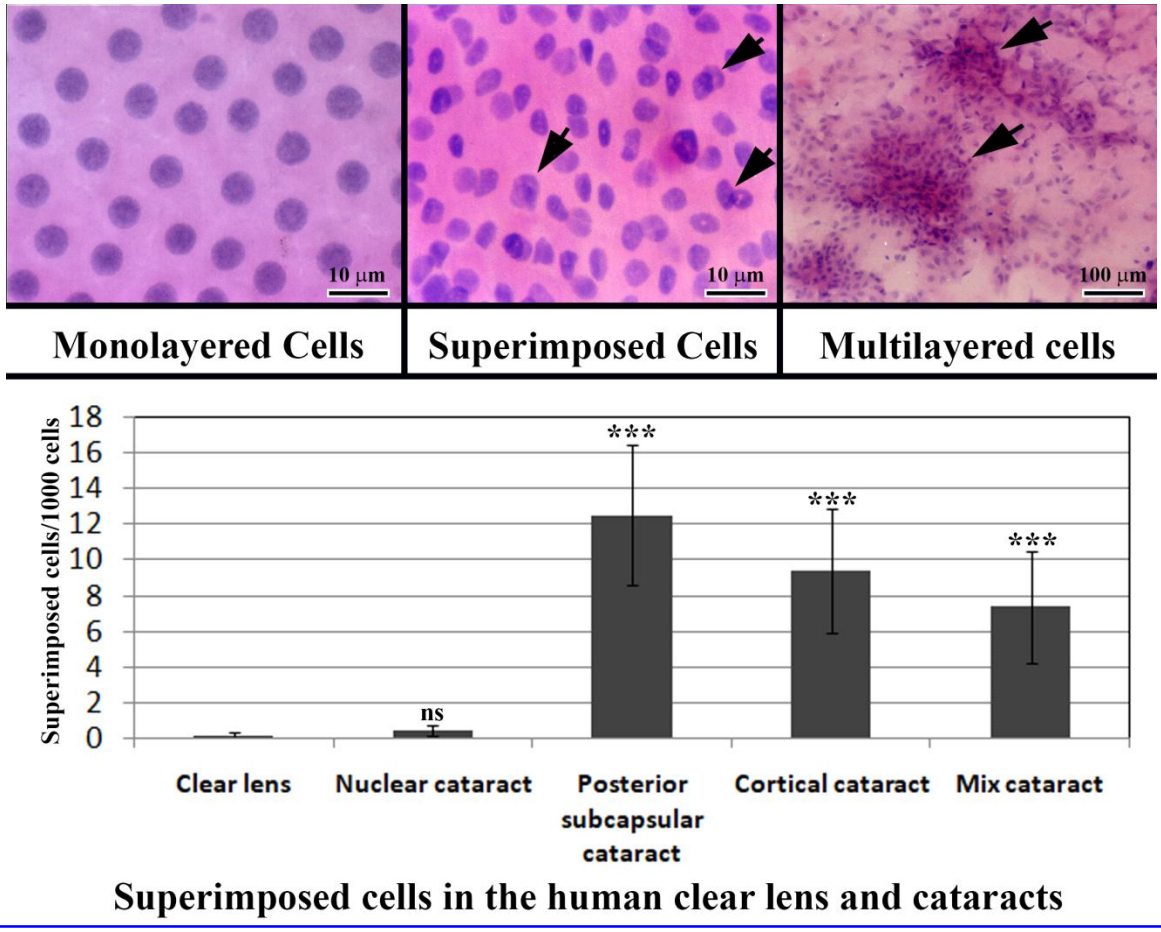


Fig. 2. Toludine blue stained semithin sections of lens epithelium obtained from clear lens and posterior subcapsular cataract indicating the presence of superimposed cells (arrow).

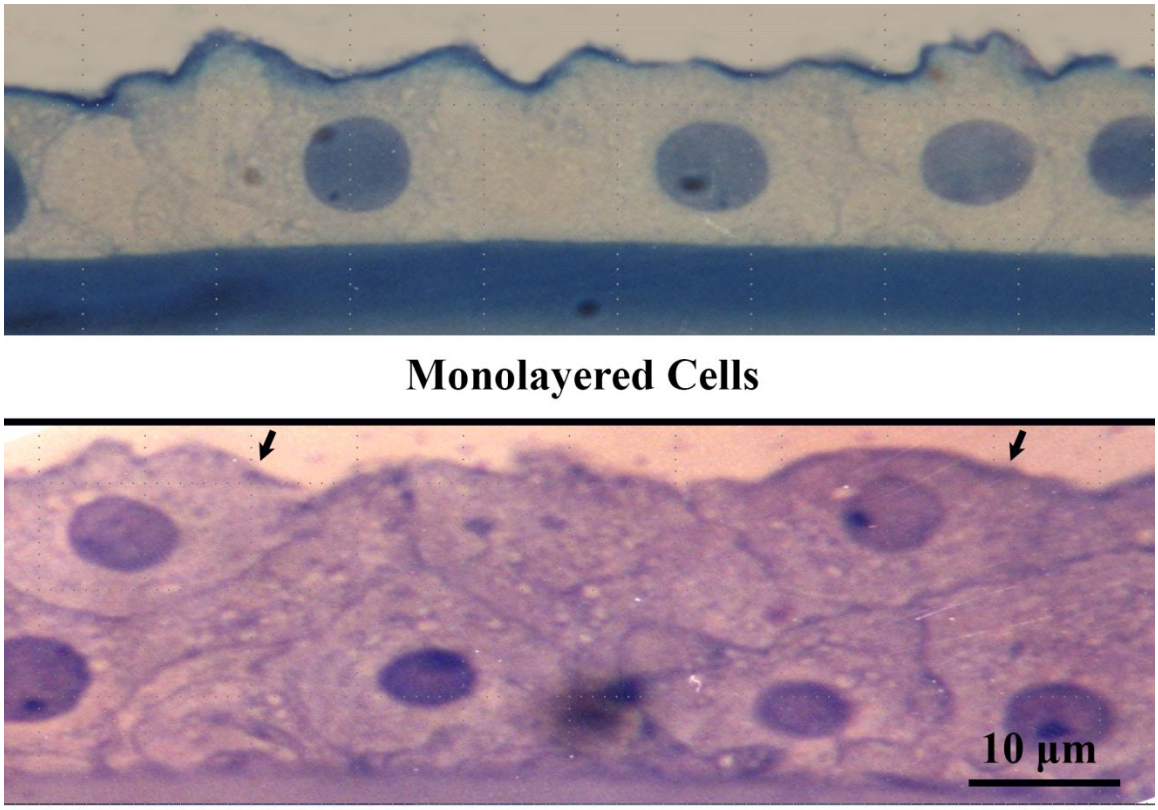
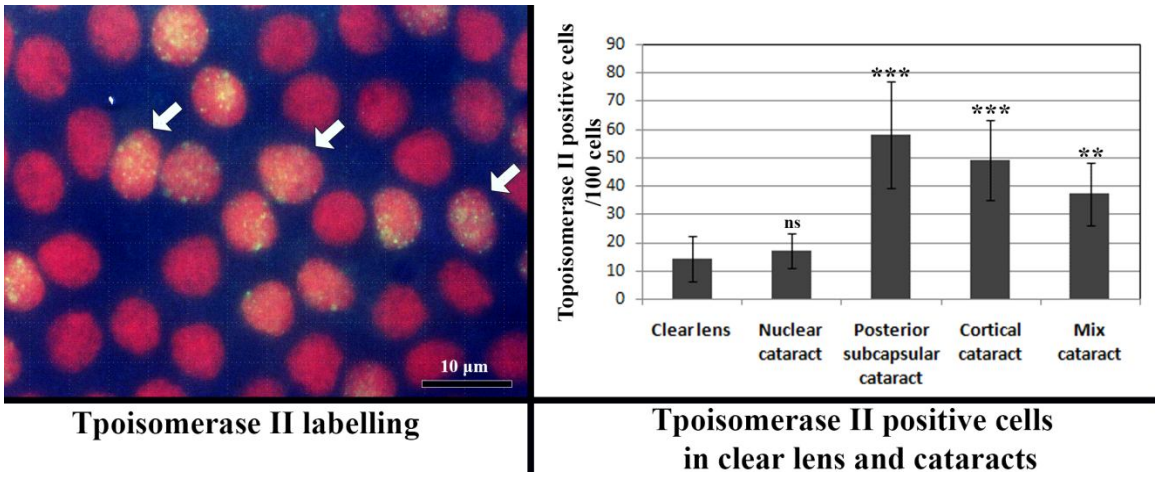


Fig. 3. Topoisomerase II positive cells (arrow) in human clear lens and different cataracts.



topoisomerase II positive proliferative cells were highest in the posterior subcapsular cataracts followed by mixed cataracts, cortical cataracts, clear lenses and nuclear cataracts (Figure 3).

DISCUSSION

Cataract in humans has been attributed to be multifactorial in origin. In experimental animal models different factors have been shown to be responsible for stratification of LECs such as oxidative insult [18], galactose [11,14], hyperension [21], UV radiation [25], smoke condensates [20], and depletion in GSH levels [15]. During the development of cataract, the LECs are invariably affected and that may lead to increased proliferation followed by extrusion of LECs from the

monolayer and subsequent superimposition of cells and multilayering. When physiologic functions of LECs are impaired, osmotic and ionic balance are disturbed and the composition of lens proteins are altered resulting in loss of transparency [8]. These types of changes in turn lead to the damage and disorganization of the lens fiber cells [27].

The LECs in the CZ of the normal human lens appeared as a uniform monolayer of nucleated cells under light microscope and TEM. This suggests that under normal conditions the epithelial cells in the CZ do not mitoses. This observation agrees with that of Harding and colleagues [28]. In our results superimposition of the LECs in the CZ has been observed in human cortical, posterior subcapsular, mature and mixed types of cataracts. This indicates that under cataractous conditions, the mitotic activity of the LECs is triggered. The stress imposed upon the LECs in the CZ during cataract formation probably acts as the signal for the G1 arrested cells prompting them to undergo cell division.

In nuclear cataract, superimposition of the LEC was not observed in the CZ. However, when nuclear cataract was present along with other cataract types such as cortical and or posterior subcapsular cataract, superimposition of the LECs was observed. This may indicate that the stress on the epithelium in the mixed cataracts may be different than that responsible for formation of pure nuclear cataract. No morphological damage or alteration has been observed in the LECs in the nuclear cataracts. Nuclear cataract is a slow progressing cataract and takes years to develop into a mature form. Probably the stress on the epithelial cells in this type of cataract may be low intensity providing time for the adaptation of the LECs to the changing environment. Other types of cataracts develop fast, within a few months and the LECs may not be able to adapt to the changes in the environment. The effect of these accumulated changes may result in the increased mitotic activity of LECs. Thus, in posterior subcapsular, cortical, mature and mixed type of cataracts, the G1 arrested cells of the CZ undergo mitotic division, which may manifest as extrusion followed by superimposition and multilayering of the LECs.

Previous studies have shown alteration in the monolayer lens epithelium and proliferation in different types of cataracts. A comparative study of the rate of proliferation of LECs obtained from 7 atopic dermatitis patients associated with atopic cataract and 1 normal human lens has also been reported [22]. It was found that out of seven atopic cataracts, LECs of only one lens showed increased proliferative activity and became multilayered while LECs from remaining atopic cataracts showed no proliferative activity. Flat mount preparations of the CZ of the LECs from patients having nuclear, posterior subcapsular, mature, mixed and black cataracts sh**o**wed the pr**e**sence of superimposed areas in the histomorphological studies [23].

Epithelial cells usually retain bidimensional monolayer organization. However, sometimes under specific conditions cells can extrude out from monolayer and later develop multilayered structure as it is observed in the skin epidermis or thymus [29]. In carcinoma, the transformed epithelial cells that express an oncogene also extrude out from monolayer in the context of epithelial mesenchymal transition (EMT) [30,31]. The fate of extruded cells depends on many factors, and it is usually context dependent. In otherwise normal tissue, the extruded cells usually undergo apoptosis as it is observed in the MDCK cells [32,33]. In physically constrained environment, cells become elongated and orient themselves in the supracellular organization of the cells [34,35]. In the ocular lens also, multilayering was observed and subsequently due to EMT, develops fibrous structure called plaque which is quite different from both the carcinoma and supracellular organizations [36]. This may be due to tight packing of cells in the ocular lens and as cells cannot escape from ocular lens due to presence of thick basement membrane (lens capsule) surrounding the lens.

CONCLUSIONS

Lens epithelial cells of posterior subcapsular, mixed and mature cataracts show superimposition of cells in the central zone of the lens epithelium. Lens epithelial cells of posterior subcapsular, mixed and mature cataracts show higher proliferative activity compared to that of nuclear cataract and clear lens.

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