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Age-related immunoreactive changes of cytokeratin 3 in the corneal epithelium and CD 31 in the corneal endothelium of buffaloes

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Abstract
The activity of cytokeratin 3 in the basal cells of the corneal epithelium increased with advancement of age, whereas the activity of wing and superficial cells was not altered with advancement of age. The activity of CD 31 was increased with age in the corneal endothelial cells of buffaloe.

Keywords: Immunoreactivity, cytokeratin 3 and CD 31 markers, cornea, ageing, buffaloes

Introduction
Keratins are a group of water-insoluble cytoskeleton proteins that form about 10 nm intermediate filaments in epithelial cells (Kinoshita *et al.,* 2001) [4]. CK3 is present in the superficial epithelial cells of the cornea and absent in the embryonic limbal cells, but after epithelial differentiation, the expression of this marker can be detected in the limbal epithelium. Enhanced expression of CK3 suggests identification and differentiation toward a mature epithelial phenotype (Jones *et al.,* 2012) [3]. Nautscher *et al.* (2015) [5] reported that CK3 positive immunoreactions was not detected in the endothelium and stroma. But strong to moderate CK3 immunoreactivity was uniformly present in the cytoplasm of superficial and intermediate cellular layers and strong reactivity in basal cells in pigs and ruminants, whereas the stratum superficiale and stratum intermedium exhibited strong to moderate activity in carnivores.
CD 31 is expressed in cell adhesive molecules in the corneal endothelial cells of the mouse cornea. The corneal endothelium was a fragile monolayer of cells with high metabolic activity, mostly represented by Na+/K+-ATPase and Mg2+-ATPase ionic pumps. The endothelium formed a leaky barrier between the aqueous humor and corneal stroma through the formation of focal tight junctions as well as gap junctions and adhesion junctions (Xie and Muller, 1996). CD 31 is expressed on all cells within the vascular compartment and on endothelial cells, where its expression is largely concentrated at junctions between adjacent cells in humans (Woodfin *et al.,* 2007) [6].

Materials and Methods
The present study was conducted on the cornea of 15 buffaloes, and samples were categorized into group I (1–5 years), group II (6–10 years), and group III (10 years above). The paraffin sections of 3–4 µm thick cornea and retina tissue samples were taken on APES (Amino Propyl Tri Ethoxy Silane) coated slides and incubated overnight at 37 ºC. These slides were subjected to an immunohistochemistry protocol (Crosby *et al.,* 2016) [2].

Table 1: Different primary antibodies used for immunohistochemistry

Sl. No	Antigen (Species)	Dilution	Target Tissue	Source
1.	Cytokeratin 3 (Mouse)	1:200	Corneal epithelium	Abcam
2.	CD 31 (Mouse)	1:500	Corneal endothelium	Abcam

Results and Discussion

The immunohistochemical studies were done on the corneal epithelium and endothelium using Cytokeratin 3 and CD 31 markers, respectively.

1. Cytokeratin 3

The cytokeratin-3 markers express the degree of maturation or differentiation within the corneal epithelium. The presence of cytokeratin 3 in the basal corneal epithelium was an indicator of cell activity during proliferation and regeneration. The immunopositive reaction of cytokeratin 3 in the corneal epithelium in group I was moderate in basal cells, whereas it was moderate to strong in group II and III buffaloes. The reaction in intermediate and superficial cells of the corneal epithelium was moderate in all age groups (Figs. 1a, 1b, and 1c). These results were almost in accordance with those of Nautscher *et al.* (2015) [5] in the cornea of ruminants and Cotinho *et al.* (1990) [1] in the human cornea.

2. CD 31

CD 31 was a cell adhesion molecule present in the endothelial cells of the body as well as the corneal endothelium. Hence, the CD 31 reaction on the corneal endothelium represents the activity of endothelial cells in the maintenance of the barrier and pump functions of the endothelium and to maintain corneal transparency, stromal deturgence, and corneal clarity. In the present study, the immunopositive reaction of CD 31 on corneal endothelium was mild in group I, moderate in group II, and strong in group III buffaloes (Fig. 2a, 2b, and 2c). Woodfin *et al.* (2007) [6] in the human cornea and Xie and Muller (1996) [7] in the cornea of mice reported that the corneal endothelium was a fragile monolayer of cells wih high metabolic activity, mostly represented by Na+/K+-ATPase and Mg2+-ATPase ionic pumps. The endothelium forms a leaky barrier between the aqueous humour and corneal stroma through the formation of focal tight junctions, as well as gap junctions and adhesion junctions. The corneal endothelium maintains corneal transparency. The corneal endothelial cells were considered nonproliferative since the rate of proliferation was not proportionate to the rate of cell loss.

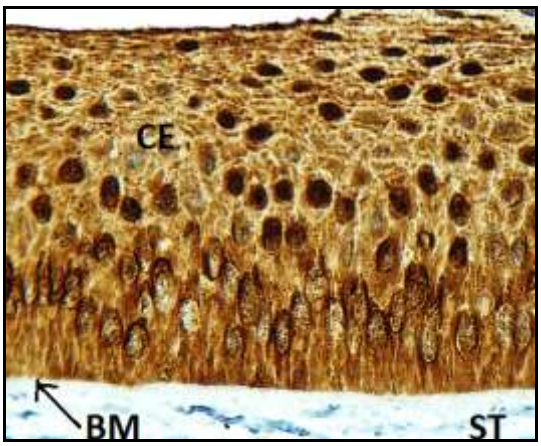

Fig 1a: Photomicrograph of corneal epithelium of group I buffaloes showing moderate immunopositive reaction for Cytokeratin3. Corneal epithelium (CE), Bowman's membrane (BM), Stroma (ST). X 400

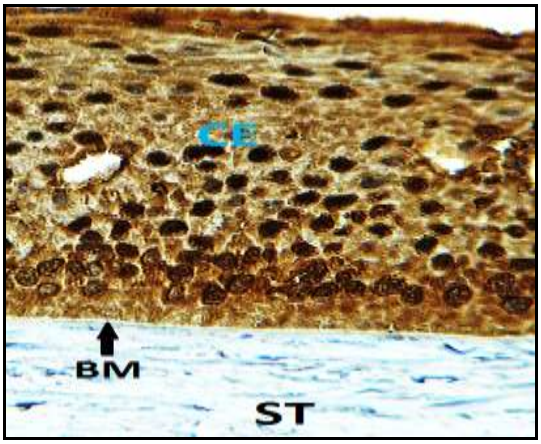

Fig 1b: Photomicrograph of corneal epithelium of group II buffaloes showing moderate to strong immunopositive reaction for Cytokeratin 3. Corneal epithelium (CE), Bowman's membrane (BM), Stroma (ST). X 400

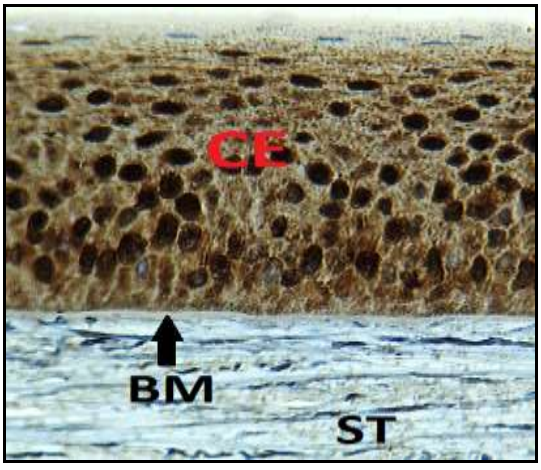

Fig 1c: Photomicrograph of corneal epithelium of group III buffaloes showing moderate to strong immunopositive reaction for Cytokeratin 3. Corneal epithelium (CE), Bowman's membrane (BM), Stroma (ST). X 400

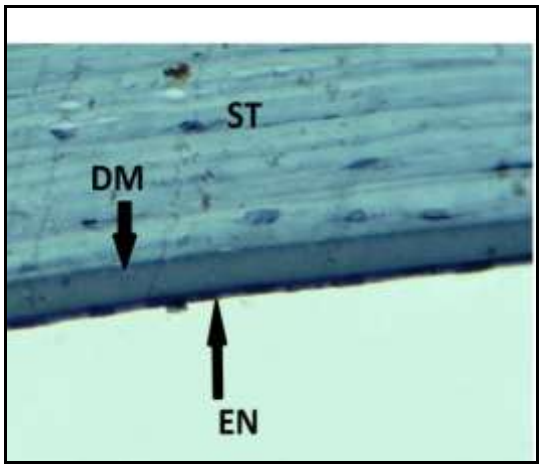

Fig 2a: Photomicrograph of corneal endothelium of group I buffaloes showing mild immunopositive reaction for CD 31. Stroma (ST), Descemet's membrane (DM) and Corneal Endothelium (EN). X 400

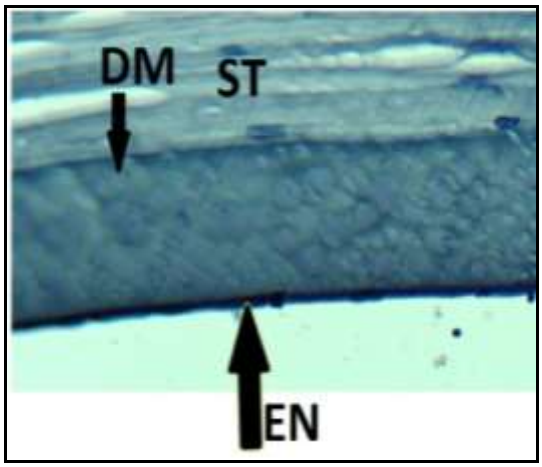

Fig 2b: Photomicrograph of corneal endothelium of group II buffaloes showing moderate immunopositive reaction for CD31. Stroma (ST), Descemet's membrane (DM) and Corneal Endothelium (EN). X 400

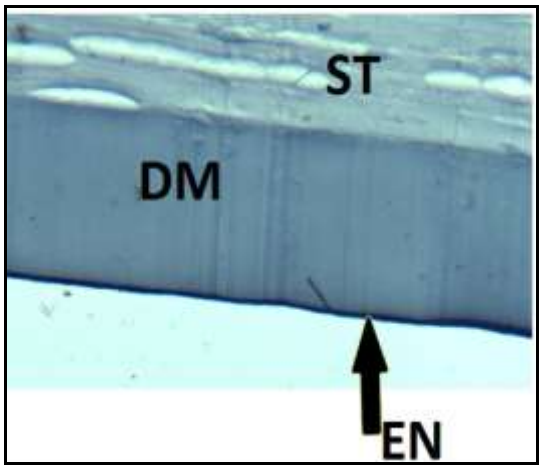

Fig 2c: Photomicrograph of corneal endothelium of group III buffaloes showing strong immunopositive reaction for CD 31. Stroma (ST), Descemet's membrane (DM) and Corneal Endothelium (EN). X400

Conclusion

The immunohistochemical reactivity of cytokeratin 3 was noted in the basal cells of the corneal epithelium. The reactivity of CD 31 was increased with age in corneal endothelial cells.

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