Age Related Ultrastructural Changes in Corneal Structure with Respect to Gender

Kornea Yapısında Cinsiyete Göre Yaşa Bağlı Ultrastrüktürel Değişiklikler

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ABSTRACT Objective: It is well known that gender plays an important role in sensitivity to various drugs, in the etiology and onset of certain diseases and it is one of the main causes leading to structural changes in various tissues. The degenerative effects of aging on some organs may vary in both sexes as well. Cornea is susceptible to the degenerative effects of aging leading to impairment of vision. The aim of the present study is to determine the effects of aging on the structure of endothelial and epithelial layers of cornea comparatively in both sexes. **Material and Methods:** In the present study, 14 male and 14 female Sprague-Dawley rats have been used. The control group was composed of 10-week-old male (n= 4) and female (n= 4) rats, and the older group was composed of 19-month-old male (n= 10) and female (n= 10) rats. Cornea samples were processed using the routine technique for transmission electron microscopical examination. **Results:** In the control group it was observed that the epithelial and endothelial layers of the cornea had a normal structure in both sexes. On the other hand, significant degenerative changes in the epithelial layer were detected in the older male group when compared to older female rats ($p< 0.05$). Distinct degenerative changes were also observed in the endothelial layer in both sexes, however there was no statistically significant difference between the sexes. **Conclusion:** In the present study it has been shown that aging causes changes in epithelial and endothelial layers of the cornea in both sexes, however the detrimental changes in the corneal epithelium is more significant in older males.

Key Words: Cornea; aging

ÖZET Amaç: Cinsiyet farklılığının bazı hastalıkların etiyolojisinde, ilaç duyarlığında, özellikle de çeşitli dokularda yapısal değişikliklerin ortaya çıkmasında rol oynadığı bilinmektedir. Yaşlanmanın bazı organlarda oluşturduğu olumsuz etkilerin cinsiyetler arasında değişkenlik gösterdiği bilinir. Kornea, yaşlanma sonucu görmeyi etkileyecek düzeyde dejeneratif değişikliklerin gelişmesine aday bir organdır. Bu çalışmada yaşlanmanın kornea epitel ve endotel katmanlarında neden olduğu dejeneratif değişikliklerin, erkek ve dişi cinste karşılaştırmalı olarak belirlenmesi amaçlanmıştır. **Gereç ve Yöntemler:** Çalışmamızda 14 erkek 14 dişi olmak üzere, toplam 28 Sprague-Dawley sıçan kullanılmıştır. Genç grup 10 haftalık erkek (n= 4) ve dişi (n= 4) sıçanlardan; yaşlı grup ise 19 aylık erkek (n=10) ve dişi (n=10) sıçanlardan oluşturuldu. Alınan kornea örnekleri rutin elektron mikroskop takibinden geçirildikten sonra transmisyon elektron mikroskopta incelenerek kornea epitel katmanındaki değişiklik ile endotel katmanındaki vakuolizasyon, mitokondriyel dejenerasyon ve intersellular aralıklardaki değişiklikler değerlendirildi. **Bulgular:** Kontrol grubunda hem erkek hem dişi cinste kornea epitel ve endotel katmanı normal yapıda gözlendi. Buna karşın yaşlı grupta korneanın epitel katmanında oluşan dejeneratif değişiklikler erkek yaşlı grupta dişi yaşlı grupla karşılaştırıldığında istatistiksel olarak anlamlı bulundu ($p< 0.05$). Endotel katmanında her iki cinste de benzer dejeneratif değişiklikler belirlendi. Ancak istatistiksel olarak birbirine göre anlamlı bulunmadı. **Sonuç:** Sonuç olarak bu çalışma yaşlanmanın her iki cinste de korneanın endotel ve epitel katmanlarında değişikliklere neden olduğunu ancak erkek cinste, dişi cinse göre korneanın epitel katının yaşlılıktan daha olumsuz yönde etkilendiğini göstermiştir.

Anahtar Kelimeler: Kornea; yaşlanma

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Several mechanisms have been asserted in an effort to explain the aging process of cells. Several studies were done on different tissues in relation to this topic. Corneal endothelium was evaluated in most of the cellular aging studies that were performed on the cornea. Corneal endothelium is situated on the inner side of the cornea and it is made up of one layered hexagonal cells.[1] Light microscopic studies show that corneal endothelium cell volume increases and cell density decreases in parallel to aging.[2] Roszkowska et al. have evaluated the morphological alterations connected with aging in the corneal endothelium and emphasized that the degeneration in peripheral regions is more conspicuous than that in the central region.[3] Similarly, an ageing dependant decrease in keratocyte density has also been observed.[3] Niederer et al. have emphasized that the ageing dependant decrease in keratocyte density is more conspicuous in the anterior part of the stroma than it is in the posterior part.[2] It has been reported that the linear loss of corneal keratocytes as a function of age is parallel to the decrease in density of endothelial cells.[2,4] On the other hand, as electron microscopical studies conducted on samples, it has been emphasized that the number of mitochondria in the front-stroma-keratocytes is roughly twice as the number of mitochondria in the central-stroma or back-stroma- keratocytes.[5] However, any age related alteration in the structure and density of epithelium cells has not been observed.[2] Jun et al. examined the effects of aging on the descement membrane had emphasized that they observed a gradual thickening of the back part of the descement membrane whereas there was no morphologic alteration or thickening in the front part.[6] Several morphometric analyses and clinical studies related to the aging-dependant alterations in the cornea conducted via confocal and specular microscope support the afore mentioned findings.[24,] However, electron microscopic studies have usually been limited to scanning electron microscope.[1] Therefore in this study it will be determined at the ultrastructural level whether the degenerations observed at the endothelial and especially epithelial layer of the peripheral cornea differ among sexes and which one is affected more severely due to aging.

MATERIAL AND METHODS

In the study, a total of 28 Sprague-Dawley rats (14 males and 14 females) were obtained and housed in Baskent University, Medical and Surgical Experimental Research Center (temperature 20 ± 2 °C, humidity 50 ± 10% and 12h light- 12h dark cycle). Rats were supplied with standard laboratory diet and tap water ad libitum. All experimental procedures involving animals were approved by the Ethical Animal Committee of Baskent University, Faculty of Medicine, Baskent Turkey (2008/AP-623; 07.07.2008). Twenty eight rats were randomly assigned to three groups: (1) Control group, ten-week old males (n=4) and ten-week-old females (n=4), (2) Nineteen-month-old male group (n=10), (3) Nineteen-month-old female group (n=10).All animals in the study were anesthetized using 60 mg/kg ketamine hydrochloride and 10 mg/kg xylazine hydrochloride. Following this process, they were sacrified. As no significant difference was noted between right and left eyes in the previous studies, right eyes of all animals were used in this study.[3] Whole globes were enucleated immediately and placed in 0.1 M phosphate-buffered containing 2.5% gluteraldehyde, for 24 hours. After incubation, corneas were excised outside the limbus, immediately postfixed in 1% osmium tetraoxide (OsO_4), for 1h 0.1 M phosphate-buffered and dehydrated in a series of graded alcohol (25-100%). After passing through propylene oxide, specimens were embedded in araldyte CY 212, DDSA (2-dodecenyl succinic anhydride), BDMA (benzyldimethyl amine) and dibutylpytalate. Semithin sections (1μm) through the peripheral cornea were stained with toluidin blue and examined under a light microscope. Ultra-thin sections of peripheral cornea were stained with uranyl acetate and lead citrate and examined with LEO 906E transmission electron microscope (TEM). For each animal, a minimum of four TEM images were obtained from four different sections obtained from the same block.

Existence or absence of the changes in the epithelial layer of cornea, vacuolisation in the endotelial cells, and dilatation in interendotelial gaps were evaluated. SPSS 12.5 was used for statistical analyses. Statistical comparison was performed using Fis-

her's exact test, since expected frequencies were low, a P value <0.05 was considered significant.

RESULTS

In this study, aging-related changes that emerge in the epithelium and endothelial layers of peripheral cornea were examined at the ultrastructural level and the results were compared for gender and age. In the younger group stratified squamous epithelium and endothelial layers of the peripheral cornea were normal in both male and female samples (Figure 1, 2). Only few rats in the older female group had degenerative changes in the epithelial layer. These alterations were as follows: Vacuolization in cytoplasms of epithelial cells nucleus degeneration in some epithelial cells, and dilation of cellular borders in epithelial cells (Figure 3, 4). However, statistical analysis showed that these alterations were not significant when compared to the young control female group (p> 0.05). Epithelial layer was found to be degenerated in almost every member of the older male group. Statistical analysis indicated that these degenerative changes showed significant difference when compared to those of the older female group (Table 1) (p= 0.035). Of these changes, especially deep invaginations accompanied by basal membrane on the basal surface of the cells located at the basal layer of corneal epithelium were very interesting (Figure 5, 6). Moreover, nuclei of many epithelial cells had degenerative alterations such as karyolysis. Although it was observed less frequently, the intercellular gap between the surface cells of epithelium layer had also been dilated. Although the epithelial layer was found normal in many older female rats, the endothelial layer of the corne-

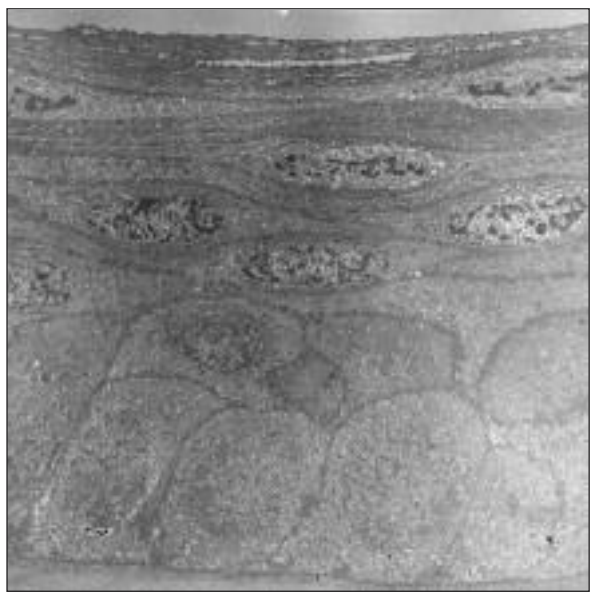

FIGURE 1: Control (young) group, stratified squamous epithelium of cornea with a normal structure (X 1670).

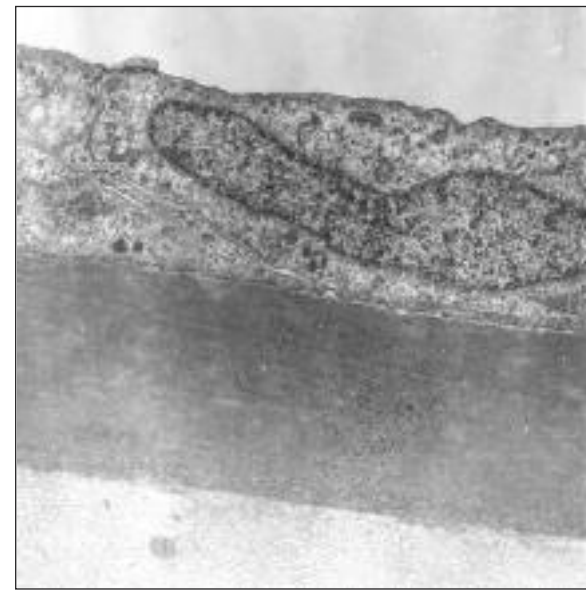

FIGURE 2: Control (young) group, endothelial layer of cornea with a normal structure (X 7750).

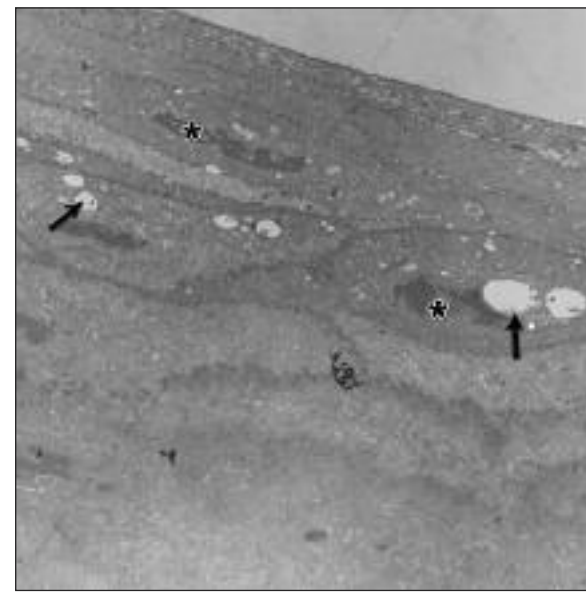

FIGURE 3: Degenerated cellular nuclei (asteriks) and vacuolization (arrow) in epithelial cells -older female group (X 2156).

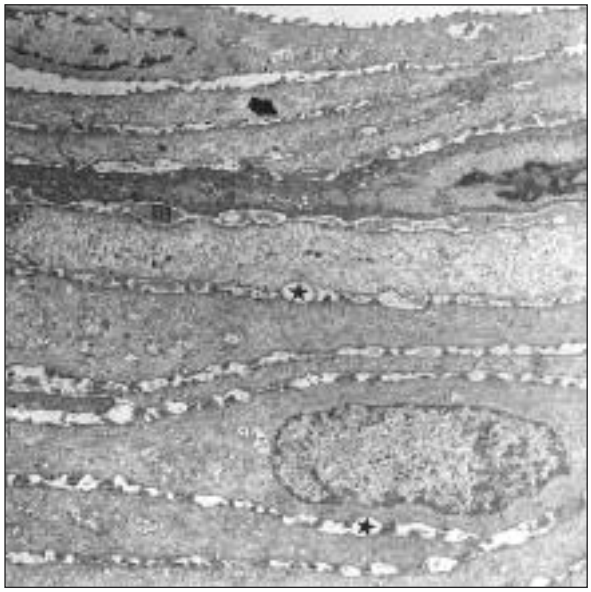

FIGURE 4: Expansion of epithelial cell borders (asteriks)- older female group (X 3597).

TABLE 1: Corneal epithelial degenerative changes in the older groups.

	Epithelial degenerative changes	
	Present	Absent-
Female	2	8
Male	7	3

P=0.035.

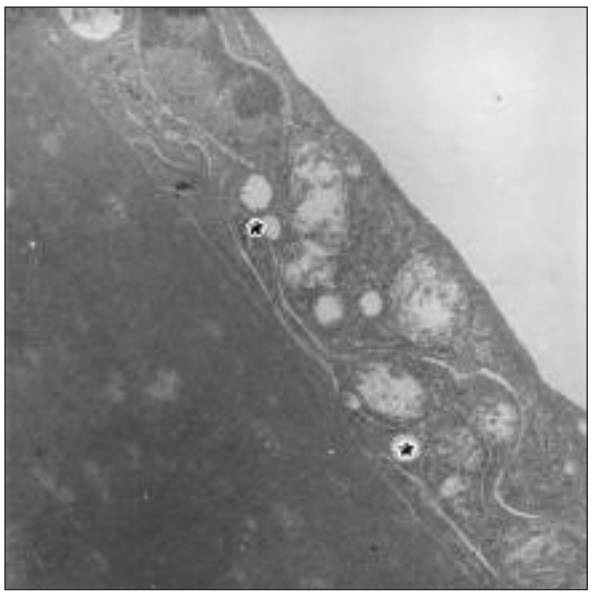

FIGURE 5: Deep invaginations (asteriks) accompanied by basal membrane on the basal surface of the cells, which are located at the basal layer of corneal epithelium – older male group (X 6000).

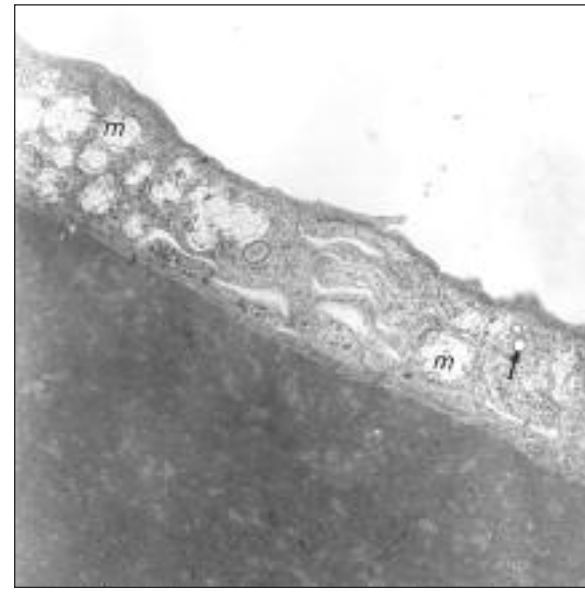

FIGURE 6: Deep invaginations (asteriks) accompanied by basal membrane on the basal surface of the cells, which are located at the basal layer of corneal epithelium – older male group (X 6000).

a was vastly degenerated in almost every member of this group. The common structural changes observed in endothelial cells were vacuolization (Figure 7), mitochondrial degeneration (Figure 8) and dilation of intercellular gaps (Figure 9). Similar to older female group, the mostly affected layer was the endothelial cell layer in the older male group. When compared to the younger group, corneal endothelial layer was extremely thinner and irregularly aligned in the older group, due to the deformation of cell structure (Figure 10). The endothelial cells of almost all of the rats in the older male group had similar alterations with those observed in the older female group: vacuolization, mitochondrial degeneration and substantial widening of intercellular gaps (Figure 11).

In both sexes, degenerative changes in the endothelial layer were grouped as vacuolization, mitochondrial degeneration and dilatation of intercellular gap. No statistically significant difference was observed between the two sexes with regard to the degenerative changes in endothelial layer due to age ($p > 0.05$) (Table 2).

DISCUSSION

It is known that particularly cells with limited proliferation capacity cannot maintain their normal

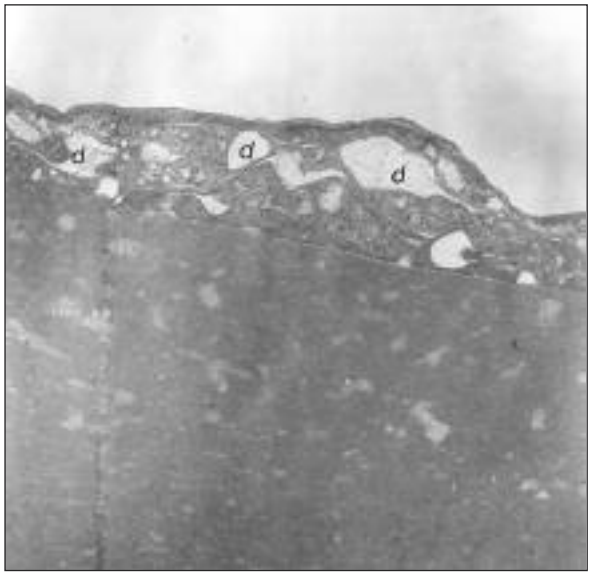

FIGURE 7: Vacuolization (asteriks) in endothelial cells-older female group (X 10 000).

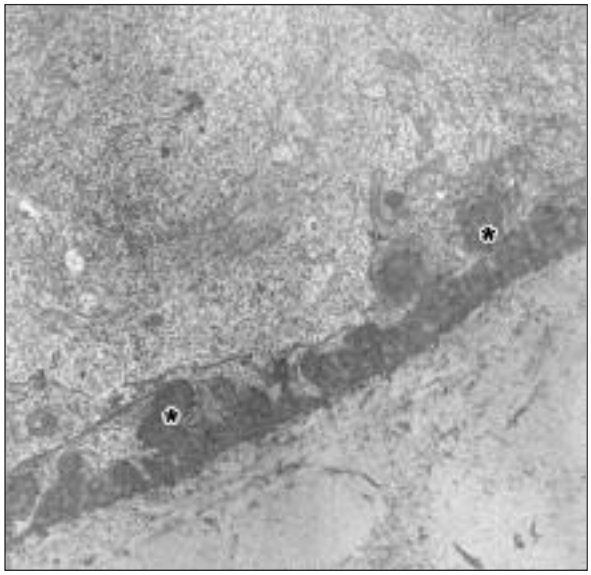

FIGURE 8: Mitochondrial degeneration (m) and vacuolization (arrow) in endothelial cells-older female group (X 6000).

and healthy forms for a long period of time.[7] Corneal endothelial cells have a limited regenerative capacity and low replication rates. In the studies conducted on man and other mammals it was observed that the rate of endothelial cell division occurrences following trauma or disease is low. [8-10]

Besides, exposure to light has a crucial role on the variation and proliferation of endothelial cells.[6] Corneal endothelial separates the aqueous humor that is situated at the anterior segment from the hydrophilic corneal stroma and it secretes the Descemet's membrane which lies between the stromal and endothelial layers of cornea. Corneal endothelium is responsible for the control of corneal hydration, and thereby maintenance of the transparency of cornea [9,11,12] Along with all its known functions, it is the layer with highest amount of aging related degenerative changes because of its low

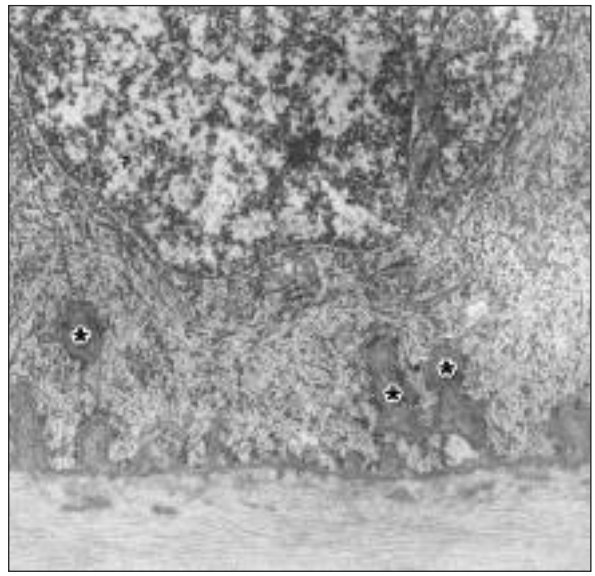

FIGURE 9: Substantial dilation (d) of intercellular gaps in the endothelial layer-older female group (X 7750).

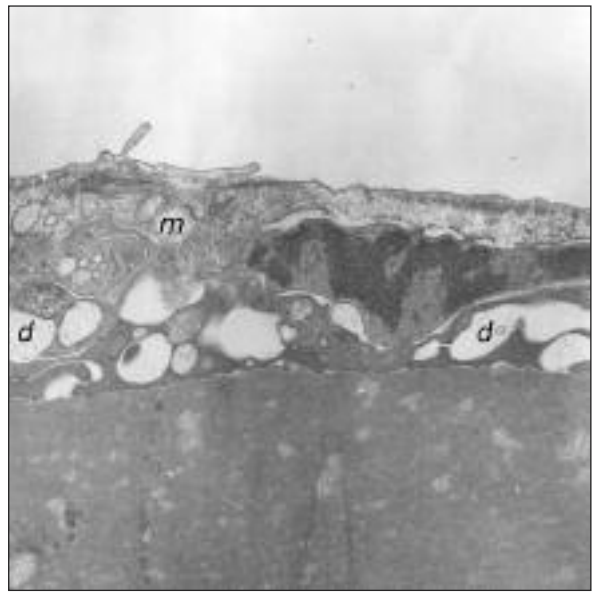

FIGURE 10: Corneal endothelial layer is extremely thinn and irregularly aligned due to the deformation of cell structure-older male group (X 3597).

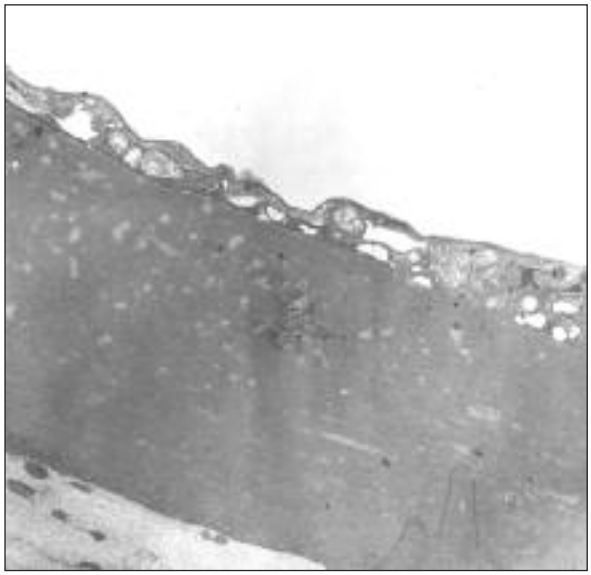

FIGURE 11: Mitochondrial degeneration (m) and dilation (d) of intercellular gaps-older male group (X 10000).

proliferation capability. The mechanism behind the aging-related endothelial cell loss has not been explicitly explained. However, it could be affected from hormonal changes, UV irritation, or from environmental changes such as chemical toxicity. Degradation of the enzymes present especially in the anterior segments which metabolize hydrogen peroxide and other free radicals cause injury on the endothelial layer.[13] Just as an organism regenerates its cells, an aging cell also renews its molecular structure and gets rid of its unnecessary organelles. Degeneration of these macromolecules and the accumulation of them in cells start the cellular aging process and kills the cell gradually.[14] Somatic mutations accumulate in the aging cell and cause the production of lots of defective proteins. As a result of this process, the density of defective lytic enzymes increases, and cellular autophagy capability decreases. Consequently, waste substances accumulate in the aging cell's cytoplasm, and in time, the ratio of these substances to the functionally active structures increases. Therefore, the elder cells have larger volumes when compared to the young cells. From the ultrastructural point of view aspect, degenerations observed in mitochondria and lysosomes are the most conspicuous changes among the alterations that occur in aging cells. Mitochondrial swelling, disappearance and degeneration of cristae (cristalysis) and excessive growth in some mitochondria can be observed. [15,16] It was also observed in this study that the endothelial mitochondria of both sexes in the control group seemed normal, on the other hand, in the older male and female group, the mostly affected organelle of endothelial cells were mitochondria. The most frequent mitochondrial degeneration in both sexes was the disappearance of the cristae.

Light microscopic studies emphasize that the volumes of corneal endothelial cells increase whereas their cellular density decrease with aging.[2] A study conducted on cats, dogs, rats, horses and humans also supported this argument, and asserted that the density of corneal endothelial cells decrease with aging. It has been reported that the decrease in the density of endothelial cells appears first with transition from childhood to adulthood, as corneal diameter increases in this period and that the density continues to decrease for the lifetime.[1,17-20]

Age-related decrease in the number of endothelial cells and changes in cell size and shape results in impaired endothelial functions. Besides, decreased functions due to aging-related decrease in the high energy metabolism decreases in the number of endothelial cells, and the variations in cellular shape and size affect endothelial functions.[21] Similarly in our study it has been observed that the change in size and shape of endothelial cells in both older male and female groups manifested itself as thinning thus irregular alignment of and the endothelium. Thickening of the Desce-

TABLE 2: Corneal endotelial degenerative changes in the older groups.

Endothelial Degenerative Changes	Dilation of Inter endothelial Cell Gaps		Vacuolization		Mitochondrial Degeneration	
	Present	Absent-	Present	Absent-	Present	Absent-
Female	9	1	8	2	9	1
Male	8	2	4	6	6	4

P=0.500, p= 0.085, P=0.152

met's membrane accompanies the decrease in the number of endothelial cells by advancing age.[6] Age-related Descemet's membrane studies conducted on two rat species (ddY and C3H) have shown that two-year-old rats showed signs of focal thickening instead of the uniform thickening observed in old people.[22] Morphological studies have shown that the density of human corneal endothelial cell is higher in the peripheral part of the cornea than it is in the central part.[23] Density of corneal endothelial cells in the peripheral and central region of young and elder donors for the post-wounding period has been analyzed. Although there was no difference between the peripheral and central regions of the young donors, markedly higher cellular density has been reported in elder in the peripheral region donors when compared to the central regions. These findings reflect either that decreased numbers of endothelial cells at the central region of the cornea have the capacity of migrating to the wounded region or that the response speed to the incidence of wounding is markedly low.[24] Corneal endothelial cell density has been studied by specular microscope in different age groups and adults with different sexes. Although no difference has been noted in cellular density with respect to sex, it has been shown that for all individuals, the endothelial cell density is higher in the central part of cornea than it is in the peripheral compartments of the same samples. It has been noted that endothelial cell density increases with aging. While the decrease in the density of endothelial cells for the young and middle aged individuals (20-44 years) has been homogenous in the peripheral and central parts, for the elder individuals (especially for the ones over 70 years of age) the decrease has shown a significant difference between the central and peripheral parts and that the loss of cells in the peripheral parts has been more conspicuous than it has been in the central part.[3]

Since aging affects the peripheral part of the cornea more severely than the central part, the peripheral part of the cornea has been used in our study. Although cells with limited proliferative capability, such as corneal endothelium, frequently show signs of aging-related degeneration, cells that divide frequently and relatively short living daughter cells that come into existence consequently degenerate much less. In such kind of cells, no apparent degeneration is observed even at the end of their lifespan.[25]

Corneal epithelium has a stratified squamous structure and regeneration time is rather short. Because of this feature, the number of aging-related degenerations is relatively low. Although no significant aging-related change in epithelial cell density has been detected in a study, it has been noted that corneal innervations reduced notably and it has been asserted that this reduction caused a delay in healing of corneal wounds.[2] No aging-related alterations have been detected in a study conducted on a small sample of normal corneal epithelium taken from elder individuals.[26] Similarly in the current study, ageing-related degenerations in the corneal epithelial layer of older female rats have not been found to be significant. On the contrary, Alvarado et al. have detected some specific aging-related alterations on their studies conducted on basement membrane of normal corneal epithelial layer taken from specimens with varying ages.[27] These changes manifest themselves as a progressive thickening either accompanied or not accompanied by reduplication on the basal membrane. This thickening has been assessed in relation with the adhesive changes in the corneal epithelium. Similarly in this study, degenerations observed in corneal epithelial layers of older male rats have been found to be more significant than those in older female rats. Of these changes, especially deep invaginations that were accompanied by basal membrane have been detected on the basal surface of the cells located at the basal layer of corneal epithelium. Moreover, nuclei of many epithelial cells have degenerative alterations such as karyolysis.

Gender-related differences are believed to play a role in the etiology of some diseases and changes in tissue structure.[28,29] For example, coronary artery disease and osteoporosis are reported to be gender-related. The risk of sudden death owing to acute myocardial infarction before hospital admission is higher in men.[24] Conversely, women are more susceptible to osteoporosis than men.[25] Although the causes of these gender- related differences are not

well understood, differences in sex hormones in men and women have been suggested.[30] Furthermore, corneal thickness has been found to increase with higher levels of estrogen in women; it decreases at the end of menses and increase again at ovulation.[31] Physiological research has shown that the interfibrillar spacing of corneal collagen decreases with age, whereas the collagen bundles become thicker.[32] These structural changes may alter the rigidity and elasticity of the cornea, then corneal irregularity increases with age. Aging-related changes in the slope of cornea make one theoretically think that some intrinsic factors just as change in ocular axial length have influence on cornea and that the possible effect can be the lack of sex hormones.[33] Sex hormone (predominantly androgen, estrogen and progesterone) receptor mRNAs have been identified in rat, rabbit and human cornea.[34] Aging decreases either the sex hormone level or functional hormone receptors in the cornea.[35] The decrease in sex hormone levels may have a great influence on the change in corneal curvature in men than in women.[36]

As a conclusion, in our study we have reached the following opinions: The reason for the similar degenerations observed in corneal endothelium in both sexes is that corneal endothelium has a limited regeneration capability. Considering the fact that both sexes lived under the same laboratory conditions, hormonal effect should play its role on females and eventually can be the answer of why degeneration of epithelial layer has been found to be more significant in males than in females.

REFERENCES

1. Rodrigues GN, Laus JL, Santos JM, Rigueiro MP, Smith RL. Corneal endothelial cell morphology of normal dogs in different ages. Vet Ophthalmol 2006;9(2):101-7.
2. Niederer RL, Perumal D, Sherwin T, McGhee CN. Age-related differences in the normal human cornea: a laser scanning in vivo confocal microscopy study. Br J Ophthalmol 2007;91 (9):1165-9.
3. Roszkowska AM, Colosi P, D'Angelo P, Ferreri G. Age-related modifications of the corneal endothelium in adults. Int Ophthalmol 2004;25 (3):163-6.
4. Møller-Pedersen T. A comparative study of human corneal keratocyte and endothelial cell density during aging. Cornea 1997;16(3):333-8.
5. Müller LJ, Pels L, Vrensen GF. Novel aspects of the ultrastructural organization of human corneal keratocytes. Invest Ophthalmol Vis Sci 1995;36(13):2557-67.
6. Jun AS, Chakravarti S, Edelhauser HF, Kimos M. Aging changes of mouse corneal endothelium and Descemet's membrane. Exp Eye Res 2006;83(4):890-6.
7. Terman A, Gustafsson B, Brunk UT. Autophagy, organelles and ageing. J Pathol 2007;211(2):134-43.
8. Tuft SJ, Coster DJ. The corneal endothelium. Eye (Lond) 1990;4 (Pt 3):389-424.
9. Gwin RM, Lerner I, Warren JK, Gum G. Decrease in canine corneal endothelial cell density and increase in corneal thickness as functions of age. Invest Ophthalmol Vis Sci 1982;22(2):267-71.
10. Padmanabhan P, Basti S, Murugesan R. Effect of two anterior capsulotomy techniques on the corneal endothelium. J Cataract Refract Surg 1994;20(5):504-6.
11. Svedbergh B, Bill A. Scanning electron microscopic studies of the corneal endothelium in man and monkeys. Acta Ophthalmol (Copenh) 1972;50(3):321-36.
12. Ringvold A, Davanger M, Olsen EG. On the spatial organization of the cornea endothelium Acta Ophthalmol (Copenh) 1984;62(6): 911-8.
13. Green K. Free radicals and aging of anterior segment tissues of the eye: a hypothesis. Ophthalmic Res 1995;27(Suppl 1):143-9.
14. Barja G. Free radicals and aging. Trends Neurosci 2004;27(10):595-600.
15. Beregi E, Regius O, Hüttl T, Göbl Z. Age-related changes in the skeletal muscle cells. Z Gerontol 1988;21(2):83-6.
16. Terman A, Dalen H, Eaton JW, Neuzil J, Brunk UT. Mitochondrial recycling and aging of cardiac myocytes: the role of autophagocytosis. Exp Gerontol 2003;38(8):863-76.
17. Wilson RS, Roper-Hall MJ. Effect of age on the endothelial cell count in the normal eye. Br J Ophthalmol 1982;66(8):513-5.
18. Meyer LA, Ubels JL, Edelhauser HF. Corneal endothelial morphology in the rat. Effects of aging, diabetes, and topical aldose reductase inhibitor treatment. Invest Ophthalmol Vis Sci 1988;29(6):940-8.
19. Baroody RA, Bito LZ, DeRousseau CJ, Kaufman PL. Ocular development and aging. 1. Corneal endothelial changes in cats and in free-ranging and caged rhesus monkeys. Exp Eye Res 1987;45(4):607-22.
20. Laule A, Cable MK, Hoffman CE, Hanna C. Endothelial cell population changes of human cornea during life. Arch Ophthalmol 1978; 96(11):2031-5.
21. Lass JH, Greiner JV, Merchant TE, Glonek T. The effects of age on phosphatic metabolites of the human cornea. Cornea 1995;14(1):89-94.
22. Hayashi S, Osawa T, Tohyama K. Comparative observations on corneas, with special reference to Bowman's layer and Descemet's membrane in mammals and amphibians. J Morphol 2002;254(3):247-58.
23. Amann J, Holley GP, Lee SB, Edelhauser HF. Increased endothelial cell density in the paracentral and peripheral regions of the human cornea. Am J Ophthalmol 2003;135(5):584-90.
24. Mimura T, Joyce NC. Replication competence and senescence in central and peripheral human corneal endothelium. Invest Ophthalmol Vis Sci 2006;47(4):1387-96.
25. Terman A. Garbage catastrophe theory of aging: imperfect removal of oxidative damage? Redox Rep 2001;6(1):15-26.
26. Taylor HR, Kimsey RA. Corneal epithelial basement membrane changes in diabetes. Invest Ophthalmol Vis Sci 1981;20(4):548-53.
27. Alvarado J, Murphy C, Juster R. Age-related changes in the basement membrane of the human corneal epithelium. Invest Ophthalmol Vis Sci 1983;24(8):1015-28.
28. Airaksinen KE, Ikäheimo MJ, Linnaluoto M, Tahvanainen KU, Huikuri HV. Gender difference in autonomic and hemodynamic reactions to abrupt coronary occlusion. J Am Coll Cardiol 1998;31(2):301-6.
29. Lindsay R. The role of estrogen in the prevention of osteoporosis. Endocrinol Metab Clin North Am 1998;27(2):399-409.
30. Harding JJ, Rixon KC, Marriott FH. Men have heavier lenses than women of the same age. Exp Eye Res 1977;25(6):651.
31. Kiely PM, Carney LG, Smith G. Menstrual cycle variations of corneal topography and thicknessAm J Optom Physiol Opt 1983;60 (10):822-9.
32. Malik NS, Moss SJ, Ahmed N, Furth AJ, Wall RS, Meek KM. Ageing of the human corneal stroma: structural and biochemical changes. Biochim Biophys Acta 1992;1138 (3):222-8.
33. Goto T, Klyce SD, Zheng X, Maeda N, Kuroda T, Ide C. Gender- and age-related differences in corneal topography. Cornea 2001; 20(3):270-6.
34. Wickham LA, Rocha EM, Gao J, Krenzer KL, da Silveira LA, Toda I, et al. Identification and hormonal control of sex steroid receptors in the eye. Adv Exp Med Biol 1998;438:95-100.
35. Ankrom MA, Patterson JA, d'Avis PY, Vetter UK, Blackman MR, Sponseller PD, et al. Age-related changes in human oestrogen receptor alpha function and levels in osteoblasts. Biochem J 1998;333(Pt 3):787-94.
36. Yılmaz N, Uçakhan OO, Kanpolat A. [Evaluation of normal human corneal tissue by in vivo confocal microscopy]. Turkiye Klinikleri J Ophthalmol 2003;12(2):76-81.