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Original article

The Effect of Aging on the Eye of Red Fox (*vulpes vulpes*)

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ABSTRACT

Background and aims. *The red fox, like other animals, experiences eye changes with aging: hardening and clouding of the lens. Aging also negatively affects the thickness of the retinal layers. Hence, the current study was aimed to aging effect of the eye tissue of an adult male red fox.* ***Methods.*** *Four healthy male red fox, two young and two adults, were selected. Animals were euthanized, eyes were enucleated, and histology and transmission Light microscopic investigations were performed, Complete Lens free amino acids estimation.* ***Results.*** *The lens analysis shows a difference in the concentration of amino acids at different ages. As between the optical microscopic examination of a change with age, as aging negatively affects the thickness of layers of the retina, as well as claiming death in the cells and widening class vessels in the choroid.* ***Conclusion.*** *This work provides the basis for further studies linking senescence to neurodegenerative retinal diseases. It can be concluded that senescence induces damage to the normal lens architecture as well as the retina.*

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INTRODUCTION

Aging is considered one of the main reasons for the changes associated with the optical device, seen as a gradual decline and functional or progressive deterioration of physiological function, including the weakness of vision loss and retinal degeneration [1]. By the late 1970s, life changed during puberty on visual function and task performance, and the underlying mechanisms behind these changes were related to aging [2]. Many of the structural and physiological changes were shown to occur in the eye, such as an increase in the crystalline lens and cataract [3], and drusen appear in many of the older retina [4]. The sensitivity of the peripheral field of vision decreases more rapidly with aging.

It has been shown that in some carnivores, especially in the red fox (*Vulpes vulpes*), the visual acuity is superior to that found among mammals. The mechanism of accommodation in the red fox is the most efficient amongst vertebrates, with an extreme range of accommodation [5,6]. Anatomically, the eyes of a red fox are characterized by several unique structures, such as the scleral ossicles, a ciliary body closely abutted on the lens capsule, and the high density of zonulae fibers. It has been reported that the eye lens is rather exceptional among vertebrates in that it is a moldable soft structure. The red fox retina shows adaptations to nocturnal activity in a forest habitat. The accommodative mechanism of the red fox is considered to be the most efficient among vertebrates [5].

Retina is a specialized sensory organ capable of transforming light into electric signals that are transmitted via the optic nerve to the visual centers of the brain. The mature vertebrate retina consists of two distinct tissues: the neural retina, composed of neurons and glial cells, and the retinal pigmented epithelium (RPE). The cells of the neural retina derive from multipotent progenitor cells, and their differentiation follows a precise chronological order that is found in many species [7]. The mature neural retina shows a highly organized structure composed of three cellular layers: the outer nuclear layer (ONL) composed of photoreceptors; the inner nuclear layer (INL) containing neurons (horizontal, bipolar, and amacrine) and retinal Muller glial cells (RMG); and the ganglion cell layer (GCL) that contains, in addition to ganglion cells, displaced amacrine cells and astrocytes. Two synaptic layers separate these nuclear layers: the outer and the inner plexiform layers. The axons of the ganglion cells converge at the exit of the optic nerve, forming the nerve fiber layer [8–10]. Aging in humans is associated with progressive and perhaps irreversible impairment of physiological

functions, including vision. Age-related macular degeneration (AMD) is a major cause of untreatable vision loss in the elderly. The origin of this disease is dependent on complex interactions between genetic and environmental risk factors [11]. Vision loss in AMD is attributed to photoreceptor dysfunction, caused by abnormalities of the retinal pigment epithelium (RPE), Bruch's membrane, or choriocapillaris. The physiological changes in the aging retina are remarkably similar, albeit less severe, to the pathologic changes in the AMD tissue. In both aging and AMD, multiple factors, including DNA damage and oxidative stress, are believed to contribute to the pathology. In light of these studies, it was hypothesized that a broader understanding of the molecular events that modulate the aging of the retina would provide insights into the pathogenesis of AMD [12]. Hence, the current study was aimed to aging effect of the eye tissue of an adult male red fox.

METHODS

Study deign and setting

This study was cross-sectional and chemical on the eye of the red fox (Vulpes vulpes). study conducted from April to May 2022 at the Laboratories of the Department of Zoology, Omar Al-Mukhtar University. All study protocols followed the Guide for Animal Experimental Protocol (MK-IACUC: 2010-0088) under the approval of the Institutional Animal Care and Use Committee of Omar Al-Mukhtar University.

Experimental work

Four male Red Foxes (*vulpes vulpes*) at young age and two as adults were selected. The young adult red foxes used in this study were male and were 8–12 months old (2700–3200 g body weight), and the aging red foxes used in this study were male and were 36–48 months old (4700–5600 g body weight). Animals were anesthetized with xylazine hydrochloride (5 mg/kg intramuscularly) and ketamine hydrochloride (50 mg/kg intramuscularly) [13]. Eyeballs were enucleated, and the crystalline lens and the retina were separated after a limbal incision was made around the cornea**.**

Light microscopic investigations: The eyes of the selected animals were separated immediately after dissection, and different parts were separated and fixed in 10% formal saline. The specimens were dehydrated in ascending grades of ethyl alcohol, cleared in xylol, mounted in molten paraplast at 58–60 °C, and cut 5µm thick. Serial sagittal sections were cut through the cornea (vertically), ciliary body, and retina. The selected tissues of the eyeball were stained in Mayer's hematoxylin and eosin and processed for investigation under a bright-field light microscope and photographed**.**

Lens-free amino acids estimation: Eyeballs of the selected specimens were dissected, and lenses were separated and hydrolyzed by 6M hydrochloric acid (24 hours, 110 °C). Sensitive amino acids (especially tryptophan and cysteine) were partially destroyed. Gas phase hydrolysis with the addition of other acids (e.g., propionic acid, methansulfonic acid) was used to shorten the hydrolysis time and improve the yield of sensitive amino acids. The samples were washed in a hot, dilute detergent solution at a neutral pH and rinsed in warm tap water and then distilled water. Any pulpy protein in the column was squeezed out and extracted several times with petroleum ether, followed by 95% ethyl alcohol, and allowed to dry in a watch glass. The samples were dried under vacuum, redissolved in 10 to 100 µl 0.2 M sodium citrate buffer (pH 2.0), and loaded on an amino acid analyzer equipped with a cation exchange column (Amersham Pharmacia Biotech), which was equilibrated in 0.2 M sodium citrate buffer (pH 2.0). Elution was performed with a gradient of pH and ionic strength as instructed by the manufacturer. The detection of the modified amino acids was achieved calorimetrically at 440 nm for proline and hydroxyproline and at 570 nm for all other amino acids [14].

Statistical analysis

Data were presented as means ± standard error (SE). The statistical analysis was performed with multi-variant analysis of variance (MANOVA) using the SPSS (version 13) software package for Windows, comparing the multivariations between each aged animal in the same group as well as among different classes. The F-test was calculated and considered statistically significant at a $p < 0.05$**.**

RESULTS

Lens: amino acid determinations

Figure (1) illustrates the amino acids content of the lenses of red foxes at different ages. In Red Fox, the amino acids contents gradually decreased with the progress of aging.

Fig (1). Lens amino acids concentrations of young and old Red Fox

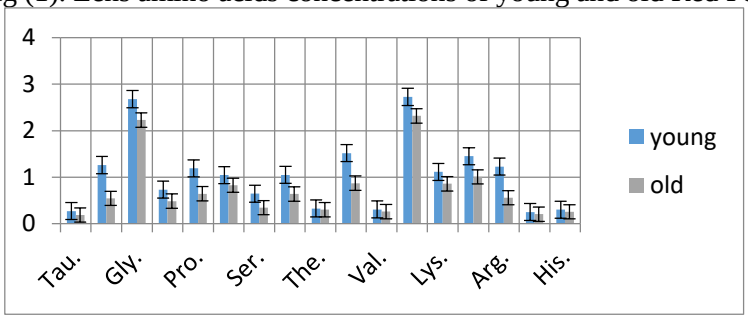

Fig (1). Lens amino acids concentrations of young and old Red Fox. The data are represented by the Mean±SE of 5 replicates (n=5). Significant at P. < 0.05. Abbreviations: Tau, Taurine; Glu, Glutamic acid; Gly, Glycine; Leu, Leucine; Pro, Proline; Ala, Alanine; Ser, Serine; Ph.Ala., Phenylalanine; The, Thereonine; Val, Valine; Met, Methionine; Lys, Lysine; Asp, Aspartic acid; Arg, Arginine; Iso.Leu; Isoleucine; His, Histidine.

Retina

Morphometric observations: Figure (2) illustrates the mean thickness of retina and the average of their cell layers of the selected ages. Comparison of the retinal thickness revealed that these folds' increase in their thickness by approximately 125.2 & 140.3 µm for young and old ages, respectively.

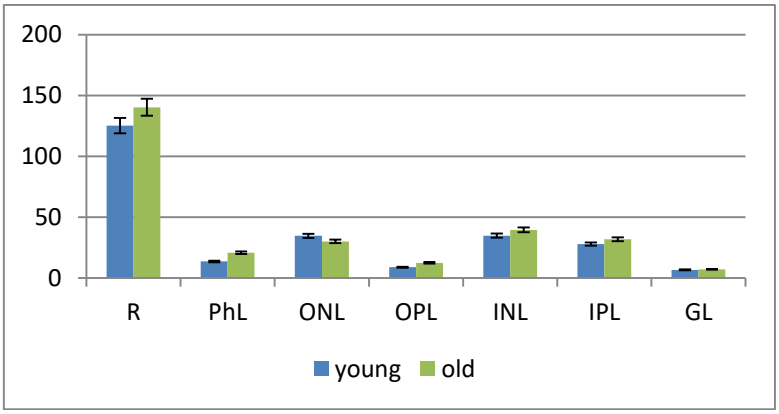

Figure 2. Mean thickness of retinal cell layers of young and old red fox. The data are represented by the Mean ±SE of 5 replicates (n=5). Significant at P. < 0.05. Abbreviations: R, retina; Ph.L., photoreceptor cell layer; ONL, outer nuclear cell layer; INL, inner nuclear cell layer; IPL, inner plexiform cell layer; GL, ganglionic cell layer.

At the light microscopic level, the retina of young exhibited characteristic pattern structures in prepared specimens with either hematoxylin eosin. The inner limiting membrane appeared enfolding. The inner and outer nuclear walls were markedly thickened. The pigmented epithelial layer was regularly arranged as a single layer of cells with a prominent basal lamina underlined by Bruch's membrane separates the retina from the choriocapillaries. The apical villous cytoplasmic processes of the pigmented epithelium showed abundant distribution of dark-borwn melanosomes (Fig. 3 A). On the other hand, in old individuals, there were considerable changes in different retinal layers. There was a considerable reduction of nerve fibers and ganglion layers. The dark-brown melanosome pigmentation appeared comparatively folded. The outer nuclear cells showed an apparent reduction and numerical loss of their cell contents (Fig. 3 B).

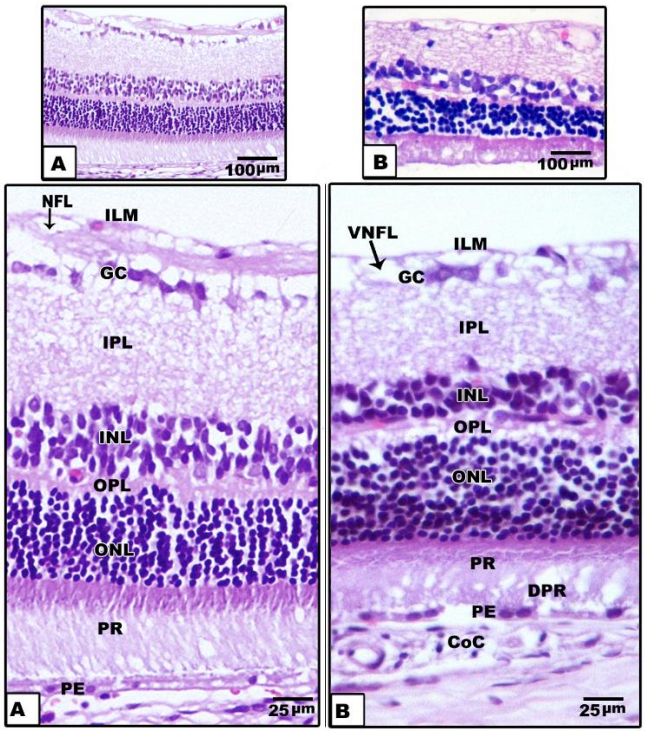

Fig.3(A). photomicrographs of transverse histological sections of retina of young and old Red fox. Both show normally compacted retinal layers; pigmented epithelium (PE),photoreceptors (PR),outer nuclear layer (ONL), outer plexiform (OPL), inner nuclear (INL), inner plexiform (IPL), gangilionic cell (GC), Inner limiting membrane (ILM) and nerve fibers (NFL). Hx-E.
Fig.3(B). photomicrographs of transverse histological sections of retina of old Red fox. Both showing considerable reduction and vacuolation of gangilionic and nerve fiber layer. There are widespread necrotic patches of compacted choriocapillaries (CoC), photoreceptors (DPR), vacuolated nerve fiber layer (VNFL) and vacuolated pigmented epithelium (PE). Hx-E.

DISCUSSION

Age-related ocular degeneration is a biological phenomenon that interferes with the structure and function of different eye components (lens, retina), leading to a reduction in visual acuity and blindness in senile individuals. There have been extremely limited studies of the aging of mammal species [14–17]. Concerning the lens, there is a marked presence of an annular pad around its center. The equatorial annular pad is formed of more visible modified lens fibers. Between the central body of the lens and the annular pad is a fluid-filled cleft, or lenticular space. This finding was consistent with Gamal et al., who reported that following the investment of the lenses of chicks, mice, rats, guinea pigs, cats, dogs, cows, and sheep, they reported the presence of a characteristic annular pad (annular ring) [18].

The presence of an annular pad in red fox species may facilitate focus on near and far by squeezing or stretching their lenses. As for the lens protein contents, earlier work was done on the lens amino acids content. Except for aspartate, valine, and methionine, the estimated amino acid increased steadily during aging [19]. The amino acids proline, serine, threonine, methionine, lysine, and arginine attained the highest accumulation level. The present findings supported the work of Hains and Truscott (2007), who showed that post-translational modifications and the accumulation of a large amount of insoluble proteins derived from soluble proteins due to aggregation are the major mechanisms in cataractogenesis. Lens cytoskeletal proteins comprise 2–4% of the total lens proteins, which include vimentin, tubulin, spectrin, actin, etc. [20]. The transparency of the crystalline lens has been attributed to the complex, ordered arrangement of its components at both microscopic and molecular levels [21]. Maintenance of the crystalline profile is essential for lens transparency, and the alteration in the elution profile in the cataract-induced group may be due to the proteolytic degradation of crystallins [22–23]. This is consistent with the earlier reports by Yan et al., which indicated that proteolysis had resulted in an increase in αH-crystalline and decrease of αL-, βH-, and βL-crystallins and the loss of many polypeptides from the soluble, insoluble, and intrinsic membrane fractions.

The estimated amino acids lysine, arginine, prolline and histidine were markedly increased during aging. These amino acids are most likely to form carbonyl derivatives as a result of direct metal-catalyzed oxidation [24]. Different explanation about the progressive decrease of protein sulfhydryls has been observed generally during the development of diabetic and senile cataracts [25-28].

During aging, the lens appears to be subjected to increasing oxidative stress manifested by decreased glutathione levels, reduced efficiency of enzymes involved in restoring reduced glutathione levels, and accumulation of proteins with di- and mixed disulfides [29]. Oxidation is considered to be a major physiological challenge to lens proteins, and recent reviews have discussed the role of oxidation in cataract [20–34]. αA-crystallin has only two Cys residues, β- and -crystallins, which are susceptible to oxidation.

The lenticular damage during subsequent aging is parallel with alterations in the retina. There is little information concerning the effect of aging on Red Fox Tinnunculus, which possessed a more characteristic increase in retinal thickness, especially in the inner and outer nuclear layers. These increased numbers of outer nuclear layers reflected the cell bodies of cones and rods and were manifested by the huge numbers of photoreceptors, which enabled more increase in visual acuity.

Red foxes possess a majority of middle-to-longwave-sensitive (M/L) and a minority of shortwave-sensitive (S) cones, indicating dichromatic color vision [35].

Observed cones contain an oil droplet rich in carotenoid pigments that acts as a filter, substantially modifying the light detected by the photoreceptor. Rod photoreceptors, specializing in vision at low light levels, are selectively vulnerable during the aging process compared with cone photoreceptors, which are specialized for vision at high light levels and for color vision [36]. Rod-mediated dark adaptation is slowed dramatically in adults, even in those with good retinal health, compared with younger adults [37]. These deficits in the steady-state and kinetic aspects of rod-mediated vision seem to have their origins in the delayed regeneration of rhodopsin, the rod's photopigment. Ohtsuka [38] identified six morphological types of cones by the color of the oil droplets located in the outermost inner segments of the red-eared turtle (Pseudemys scripta elegans). Single cones containing either red or pale green oil droplets were sensitive to red light, cones with yellow oil droplets to green, and cones with clear oil droplets to blue.

During senility in the red fox, there was a progressive deterioration of the oil droplet as well as the presence of lamellated lipofuscin in the apex of the cone's outer segment in association with considerable atrophy of the cone's outer segment. These may reflect the loss of defense against intense light exposure and aging retinal cell loss. However, in Rattus novergicus, the aging defects manifest by degeneration of the inner stacked membranes of the rod's outer segment. The observed findings revealed a massive loss of retinal gangilion cells and a reduction of nerve fibers. Rods, cones, and photosensitive retinal ganglion cells (pRGCs) were found to decline with age [39]. Age-related losses in retinal nerve fiber layer (RNFL) thickness have been assumed to be the result of an age-dependent reduction of retinal ganglion cells [40]. In old age, there was a characteristic retinal degeneration leading to reductions in rod and cone numbers and the loss of RGCs [41–45]. Harwerth et al. (2002) reported that it is reasonable the degree of vision loss would be proportional to the amount of ganglion cell loss.

CONCLUSION

The summary of this research is that with age, changes occur in the Tier natural amino acids constituting the lens, causing impairment in its function. This may be due to some metabolic products of the interactions, which free oxygen inside the cells and cause harm. This may also be the reason for the damage that occurred in the retina, where it was observed in the death of the cells making up the layers of the retina as well as the lack of thickness of these layers.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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