Histological Study of Falcon's Retina Eye and Optic Nerve

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Abstract
Background: The eye is one of the most important sensory systems in the body because of how important and relevant it is, and it is essential for communication between animals and their environment. The size of the optic nerve is thought to be closely correlated with activity pattern and is crucial for the transmission of messages between the retina and the brain. Objective: a falcon has high-resolution vision and could fly for long-distance and capture its prey quickly and accurately, so we need to detect nature of retina and optic nerve of a falcon. materials and methods: We gathered adult, healthy falcons (Falco columbarius) from Iraq, and using Periodic Acid-Schiff Reagent and hematoxylin and eosin stains and to prepare histological section also used ocular lens to measure thickness of retina and optic nerve. results: histological: by using H&E stain retina of falcon consist of ten layers as other birds but there are some little different in composition of these layers. optic nerve consists of ganglionic cell axons in the retina and are made up of a number of fascicles or bundles that are connected by vascular connective tissue septa; and by using PAS to detect glycogen in layers of retina , not all these layers contain glycogen just only two layers. in optic nerve have been founded glycogen in cells among fascicles of optic nerve. and have differences in diameter of both retina and optic nerve. Conclusions: retina and optic nerve are similar to another birds with some differences in its thickness and content of glycogen.
Keywords: falcon, retina, optic nerve, H&E, PAS, glycogen

1. Introduction

One of the body's most vital sensory systems, the eye is crucial for communication between animals and their environment [1].
Fish, amphibians, reptiles, birds, and mammals have more complex eyes than invertebrates because they use them to provide extremely sensitive vision, which makes invertebrates' eyes simpler to structure because they function to distinguish between the environment whether it is illuminated or dark [2].
Avian species have evolved very sensitive visual systems that are adapted to their living environments during more than a hundred million years of development [3]. They are more adept than mammals at detecting tiny moving objects and identifying targets in low-light conditions. For instance, raptors flying thousands of meters in the air may quickly spot tiny birds [5] and prey moving quickly on the ground [4].
Bharti (2010) displayed that the position of the eye in the head affects vision. When the eyes are located in the face area on the front of the head sides, they give monocular vision, as they give a three-dimensional image as in humans, but when the eyes are located on both sides of the head, each eye sees the visual field facing it, giving binocular vision, as in some fishes [6].
When the eyes are located on both sides of the head, each eye sees the visual field facing it, giving binocular vision, as seen in several fishes, reptiles, amphibians, birds, and mammals [6],[7].
All birds have similar eyes in terms of fundamental makeup, but there are a few variations that reflect their environmental demands [8].
A variety of factors affect how sensitive raptor eyes are in their absolute state. The ability to see well in low light is diminished in diurnal species, particularly accipitriforms and falconiforms, due to high temporal and spatial resolution, the filtering properties of cone oil droplets, low rod densities, and the absence of double cones and rods in the fovea [9].
In 2009; Hall *et al.* displayed that the optic nerve in vertebrates is the only way for vision information from the RGCs to reach the brain. [10].
The optic nerve is made up of several components, the most important of which are myelinated nerve fibers, also known as axons (white matter). The optic nerve's axons are extensions of the retinal ganglion cells, whose unmyelinated axons make up much of the neural retina's nerve fiber layer. The axons, or "nerve fibers," make a sharp bend and enter the optic disc, There they continue as a succession of fascicles or bundles that are separated from one another by septa made of vascular connective tissue and helical columns of glial cells (astrocytes). [11].
The optic nerve, the second of twelve pairs of cranial nerves, really belongs to the CNS rather than the PNS since it develops from an outgrowth of the diencephalon (optic stalks) during embryonic development. As a result, rather than the peripheral nervous system's Schwann cells, the optic nerve fibers are encased within the meninges and

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myelinated by oligodendrocytes [12].

2. Material and Methods

The birds were anesthetized with chloroform, the eye was removed from orbit with taking a part of the optic nerve, than washed with a physiological solution, and moved to a petri dish with filter paper in a physiological solution [13].

According to Al-Attar *et al*. (1982) [14] the slides were prepared, by using Hematoxylin and Eosin (H&E) Stain [15] [16] and by using Periodic Acid-Schiff Reagent (PAS) [17] [18]. The method of employed to prepare this stain through using Periodic Acid solution and Schiff reagent solution.

Have been prepared Harris' Hematoxylin Stain as a following : 10 cc of 100% ethanol were used to dissolve one gram of hematoxylin powder. Hematoxylin was dissolved in 200 ml of distilled water, 20 grams of potassium alum (KAl (So4)2.12H2O) was added, and the mixture was then boiled for 2 minutes. Mercuric oxide, 0.5 grams, added to the mixture. quickly cooled and 5 cc of glacial acetic acid were added. Before used, filter the solution. Alcoholic Eosin stain is made by combining 1 g of eosin powder with 100 ml of 70% ethyl alcohol, mixing, and adding a few drops of glacial acetic acid [15].

To prepare Periodic Acid solution add 1 gm Periodic acid to 200 ml distilled water. While prepare Schiff reagent solution add 1 gm of carbol fuschin to 200 ml of boiling distilled water and leave the solution to cool until reach 50°-60°C. then add 20 ml of 1N HCl at room temperature and add the activated charcoal. The prepared solution should keep in dark container or bottle and place over-night and filtered before using [17].

Fixation: After dissection of the optic nerve and retina, the specimen was placed in labeled tubes filled with aqueous formaldehyde. Dehydration: For 30 minutes, the specimens were submerged in increasing concentrations of ethanol (70, 80, 90, 96, and 96%). Clearing: For three times as long, the dehydrated specimens were cleared with xylene. the cleared specimens were infiltrated with pure paraffin (60 o C melting points) in the oven for 30 minutes three times, after which the specimens were embedded with paraffin by "L"-shaped templates for making labeled blocks, trimming, and sectioning: the blocked specimens were then cut with a sharp scalpel, brought out to a water bath at 50 o C for flattening, and put on microscopic slides coated with a thin layer of Mayer's albumin., Staining: Paraffin sections were used for the histological study; hematoxylin and eosin (H&E) were used to diagnose the histological architectures, and periodic acid Schiff (PAS) was used for the study of histochemistry. Mounting: The prepared microscopic slides were mounted with Dextrin plastisyzer xylene (D.P.X.), and they were then placed on the hot plate at 37°–40°C.

Ethical approval: The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 7/27/7261 IN 16/10/2022 to get this approval.

3. Results

Histological study

Eye

The following 10 layers of the retina were identified in a longitudinal segment after being stained with H&E stain, in order of proximity to the vitreous body: External limiting membrane, photoreceptors layer, retinal pigment epithelium, inner plexiform layer, inner nuclear layer, retinal ganglion cells layer, nerve fiber layer, and inner nuclear layer. (Fig. 1).

Optic nerve

The results of H&E staining revealed that the longitudinal section of optic nerve consists of ganglionic cell axons in the retina. It has various properties that distinguish it from other nerves. The axons are made up of a number of fascicles or bundles that are connected by vascular connective tissue septa. A falcon optic nerve was thick and have a high number of fibroblast (Fig. 2).

Eye

The results revealed different pattern in distribution of glycogen in retinal layer. a falcon has high content of glycogen in outer segment of rods and cones and in external limiting membrane (Fig. 3).

Optic nerve :

Longitudinal sections staining by PAS showed optic nerve in a falcon had glycogen in astrocyte and in microglia (Fig. 4).

Morphometric Study

The result of retinal thickness in a falcon was (195.5 ± 1.509 μm) while

optic nerve diameter of was (271± 0.210 μm) with significant differences.

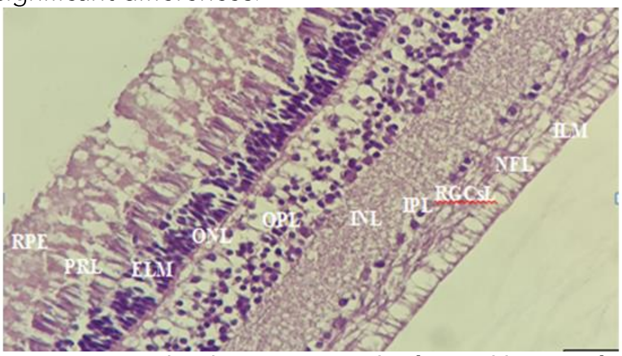

Figure (1): Light photomicrograph of retinal layers. of falcon, There are ten layers (from inside to outside) ILM: Inner limiting membrane, NFL: Nerve fiber layer, RGCsL: Retinal ganglion cells layer, IPL: Inner plexiform layer, INL: Inner nuclear layer, OPL: Outer plexiform layer, ONL: Outer nuclear layer, ELM: External limiting membrane, PRL: Photoreceptors layer, RPE: Retinal pigment epithelium. H&E, 40X.

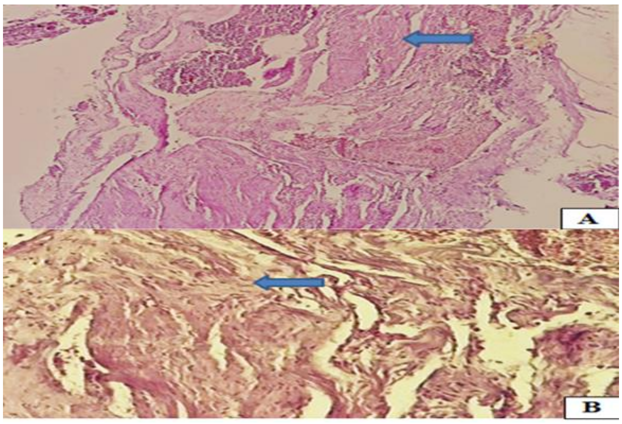

Figure (2): Longitudinal section of optic nerve of a falcon, showed high density of fascicles (A) and fibroblast (B) (blue arrow).H&E, 40X.

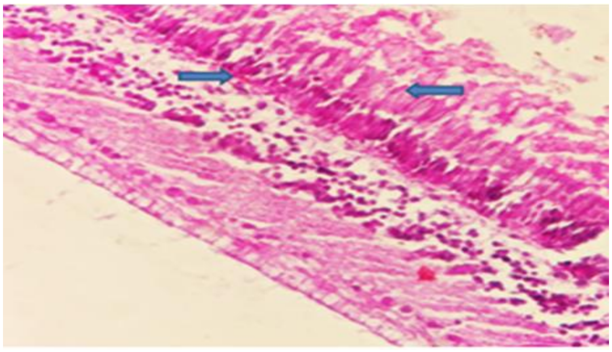

Figure (3): Longitudinal section of retina of falcon, explain distributed and high density of glycogen (blue arrow). PAS stain, 40X.

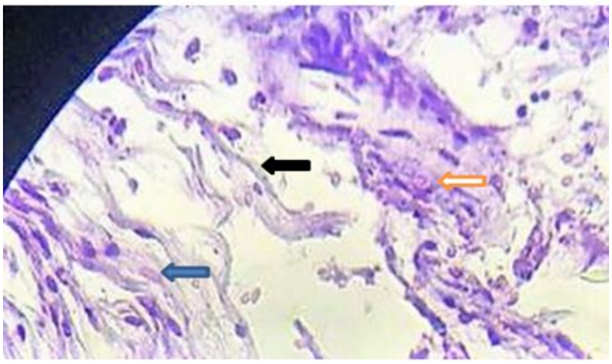

Figure (4): Longitudinal section of optic nerve of a falcon, explain distributed and density of glycogen in astrocyte (blue arrow) and microglia (orange arrow). PAS stain, 40X.

4. Discussion

First layer is Inner limiting membrane ILM: Basement membrane separate retina from vitreous body, this layer was definer and dense [19]. The inner limiting membrane and the nerve fiber layer (NFl) are separated by a thin layer of Müller cell footplates, which formed the second layer and was defined by the axons of the ganglion cell bodies[20]. RGCs in the third layer of the retinal contain the nucleus of ganglion cells, whose axons develop into the fibers of the optic nerve [21]. The results of the present study in agreement with that obtained by El-Beltagy (2015) [19] and Alivisatos *et al.* (2017) [20] who discovered that the ganglion layer, which lies between the inner plexiform layer and the nerve fiber layer, is made up of ganglion cells. There are synapses between the axons of bipolar cells and the dendrites of ganglion and amacrine cells in the inner plexiform layer (IPL). Masland agreed with the outcome (2012) [22] that describe dendrites of retinal ganglion cells (RGCs) receive synaptic input from bipolar and amacrine cells in the inner plexiform layer during normal visual processing. This data is combined, processed, and transmitted to visual areas in the brain through RGC axons in the optic nerve and this is fourth layer.

Inner nuclear layer INL: it contains nuclei and cell bodies of amacrine cells, bipolar cells, and horizontal cells are found here, it was density, Our result was collaborated with El-Beltagy (2015) [19] whom found the inner nuclear layer lie between inner plexiform layer and outer plexiform layer this is fifth layer.

Outer plexiform layer OPL: This is a thin layer that makes synapses between dendrites of bipolar cells and horizontal cells and between photoreceptors (rod and cone) and bipolar cells This result was coinciding with result of present study [23]. atomic outermost layer Cell bodies (rods and cones) made up this layer, which was moderately dense. A layer that divides the photoreceptors' inner segment regions from their cell nuclei is known as the external limiting membrane (ELM). The outer nuclear layer (ONL) has two different types of photoreceptor nuclei as a consequence of the current investigation [20]. Diurnal animals can have great acuity because of the large eyes of raptors [24]. Rod and cone inner and outer segments make up the photoreceptor layer (PRL). There is a very sophisticated light-sensing device inside the outer segments. As a result, rod and cone PRL are present in birds [25]. Rods make up less than 20% of photoreceptors in the retina of some diurnal species [26]. The retinal pigment epithelium is composed of a single layer of cuboidal epithelial cells (RPE). For clear vision, the black pigment melanin in the pigment layer blocks light reflection across the globe of the eyeball. The neural retina is supported and fed by this layer, which is the closest to the choroid. resembled our discovery in that it was made up of a single sheet of low cuboidal epithelial cells with nuclei in the center. Bruch's membrane connects this layer to the choroid [19]. Like our results, the optic nerve is a bundle of nerve fibers that transmit messages from the eye to the brain's relevant areas and vice versa [27].

As the axons of retinal ganglion cells, optic nerve fibers can convey information about an animal's visual acuity and sensitivity capabilities. Most avians do not have consistent optic nerve fiber counts [28]. Unmyelinated axons make up a large portion of the nerve fiber layer of the neural retina. The axons, also known as "nerve fibers," make a severe bend as they enter the optic disc, where they split into fascicles or bundles to create the optic nerve, which is divided by helical columns of glial cells (astrocytes) and septa made of vascular connective tissue [11]. We thought the falcon and owl's optic nerves were similar to other vertebrates except in a number of a bundle of nerves and types of cells.

Glycogen levels in the retina fluctuate when glucose levels in the medium change [29], [30]. Another research demonstrates the existence of glycogen deposits in rods, and a further peculiar characteristic seen inside the inner segments of the photoreceptors of the booted eagle is the accessory member of the double cones [31]. Depending on our findings, we thought that the distribution of glycogen depending on how much the retina of falcon was used glycogen, because of Glutamate was activated at night more than day.

This model had the benefit of being devoid of synapses and neurotransmitters, as it was made up of cable-like myelinated axons, astrocytes, and oligodendrocytes [32]. At the optic nerve, there was a lot of glycogen activity. According to glycogen's properties, it exhibits a bright red purple color of activity in both endothelium and basal lamina regions of capillaries [33].

Glycogen, which is found in the astrocytes of the optic nerve, was discovered to be the energy store [34]. Furthermore, axon conduction may be observed in real time, allowing the role of critical chemicals to be examined by careful addition or removal of energy substrates and other compounds in CSF that super fuses the tissue [35].

Birds' retinal layers are similar to those of other vertebrates; however there are some differences in morphology, visual acuity, and retinal vascularization. Furthermore, differences between diurnal and nocturnal birds are seen as a result of the interaction between environment, visual perception, and behavior. The spatial resolution of vision is determined by the size of the retinal image and the number of image points transmitted from the retina to the brain through the optic nerve [36].

Other studies have been done on other retina's birds like owl, duck and pigeon that refer to different arranged thickness of retina [37] [38]. Also another studies have been showed arranged thickness of retina depending on thickness of its layers [39] [40] [41] [42].

In vertebrates, the optic nerve is the only method for vision information from the ganglion cell layer of the retina to go to the brain. It is thought that the size of the optic nerve is highly connected with activity pattern and that the size of the optic foramen in many birds is equivalent to the size of the optic nerve. Because retinal pooling differs between nocturnal and diurnal species, nocturnal animals should have smaller optic foramina than nocturnal species. Retinal ganglion cell axons make up a large percentage of the optic nerve, which passes via the optic foramen [10].The spatial resolution ("sharpness") of vision is determined by the size of the retinal picture and the quantity of image points transmitted from the retina through the optic nerve to the brain [36].

5. Conclusions

Histological study results with hematoxylin and eosin showed that retina consists of 10 layers in a falcon. optic nerve of falcon consist of the retina's ganglionic cell axons which are divided into many fascicles or bundles and are joined by septa of vascular connective tissue. Histochemical study, PAS results showed in retina of falcon, there was high content of glycogen in outer segment of rods and external limiting membrane while in optic nerve the glycogen found in astrocyte and microglia. Morphometric study showed differences in diameter of optic nerve and retina diameter.

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