Research in: Agricultural & Veterinary Sciences
Vol.6, No.2, 2022, pp.94-103

HISTOLOGICAL EVALUATION OF AVIAN OPTIC NERVE

Hatice Yaren Kuloglu*

Aksaray University, Aksaray, Turkey

Abstract. Nervus opticus (Cr2) is a cranial nerve that carries the sense of sight and has a dense number of fibers and important neighbors. Cr2 enables sight by receiving the sense of sight perceived by the photoreceptor cells in the retina and transmitting it to other parts of the visual pathways. In our study, 24 nervus opticus (Cr2) tissue samples were taken from 12 (6 males, 6 females) chukar partridges (*Alectoris chukar*). Triple, Hematoxylin Eosin (H&E), My-Grünwalt Giemsa, Well stain and Gordon Sweet staining methods were applied to the tissue samples. The information about the general structure of the nerve opticus was obtained in the first three staining methods. These are: The dura mater and arachnoid mater were observed at the outermost, while the pia mater, which tightly encircles the nerve, was observed at the bottom. Astrocytes extensions were seen between the septa and nerve fibers. The extrafascicular connective tissue was in the same structure as the pia mater and it partially surrounded the fascicle in many places. An intense vascularization was also seen in the connective tissue. In Well Stain staining, myelin sheaths surrounding the nerve fibers were observed. In Gordon-Sweet staining, it was observed that reticular fiber bundles started from the connective tissue capsule surrounding the organ and spread by branching. These reticular fiber bundles extended into the center of the tissue in thin branches. It was observed that the reticular fiber bundles in the areas where the connective tissue septums expanded were thicker and formed a network. Reticular fibers were also found around blood vessels. In the Well Stain staining, myelin sheaths were observed around the nerve fibers in the sections after the lamina cribrosa.

Keywords: *Avian, Nervus opticus, histology, partridge, Alectoris chukar.*

Corresponding Author: *Hatice, Yaren Kuloglu, Department of Histology and Embryology, Faculty of Veterinary, Aksaray University, Aksaray, Turkey, Phone: +903822882888, e-mail: haticeyyaren@gmail.com*

Received: *15 June 2022;* ***Accepted****: 19 August 2022;* ***Published:*** *31 August 2022.*

1. Introduction

Nervus opticus (Cr2) is the second of 12 cranial nerves known as Nervi craniales (encephalici) and transmits visual information from the retina to the brain. Since it originates from embryonic retinal ganglion cells, which is a diverticulum located in the diencephalon, it cannot regenerate after being cut (Oğul, 1996; Anderson, 1969).

Nervus opticus (Cr2) carries special somatic afferent nerve fibers related to the sense of sight. Afferent nerve fibers originate from multipolar ganglion cells in the retina. There are also a small amount of efferent fibers, but their origin is not clear. Cr2 contains an average of 1,200,000 fibers. Nervus opticus (Cr2), which is approximately 4 cm long, consists of four parts: pars intraocularis (1 mm), pars intraorbitalis (25-35 mm), pars intracanalicularis (7 mm) and pars intracranialis (10-15 mm) (Arıcı & Elhan, 1997; Williams *et al*., 1995; Snell, 2000; Radius, 1994). Nervus opticus is surrounded by the cerebral cortex. In other words, the "Dura mater", "Arachnoid" and "Piamater" layers that make up the cerebral cortex also surround the optic nerve. The retinal nerve fiber layer gradually becomes thinner and extends from the optic disc to the periphery of the retina. If nerve fibers forming the visual pathways in the optic nerve are damaged in any region

for any reason such as tumor, cut, bleeding, the damaged area cannot transmit bio-electrical nerve impulses forward. As a result, since the brain cannot perceive the stimuli of the retina passing through that region, it cannot see a certain region of the visual field of the eye (Duus, 2001). Therefore, knowing the cellular organization of the optic nerve very well is very important in the precautions to be taken against optic nerve damage.

Although there is sufficient information about mammalian optic nerve histology in the literature, there is no information about avian optic nerve histology. Therefore, in this study, it was aimed to determine the optic nerve histology and histometry of chukar partridge (*Alectoris chukar*).

2. Material and Method

Animal

In this study, nervus opticus of 12 adult (6 male, 6 female) chukar partridges (*Alectoris chukar*) purchased from a commercial farm were used. The animals were kept in natural light, humidity and temperature conditions and allowed to consume water and food freely.

Histological Method

Nervus opticus tissue samples, which were taken as total, were determined by keeping them in 10% neutral formaldehyde for 24 hours. Tissue fragments were blocked in paraffin following the known histological techniques such as washing, dehydration and polishing processes. 5 μm thick sections taken from the blocks were subjected to the following procedures.

1-Triple staining technique of Mallory (Mallory, 1900) modified by Crossman for general histological examination.

2- Hematoxilin Eozin staining for general histological examination.

3- My-Grünwalt Giemsa for general histological examination.

4- Well Stain method for the demonstration of myelin sheath.

5- Gordon Sweet (GS) staining method for the demonstration of reticular fibers (Lillie & Fullmer, 1965).

The obtained preparations were examined by light microscobe (Leica DM2500, Switzerland), and the shapes of the required sites were taken with the camera attachment the same microscope Leica DFC 320 (Switzerland).

3. Result

The dura mater and arachnoid mater were observed at the outermost, while the pia mater, which tightly encircles the nerve, was observed at the bottom. Partial enlargements were noted in the subarachnoidal distance. It was observed that the pia mater completely encircled the nerve, extended towards the center and formed septal extensions that encircled the fascicles. These septa locally merged and surrounded the fascicles (Figure 1). It was observed that venules were common under the pia mater. Astrocytes extensions were seen between the septa and nerve fibers. It was determined that Cr2, which was surrounded by the pia mater, consisted of many fascicles. Nerve fibers were located within the fascicles. The extrafascicular structure composed of connective tissue between the fascicles was evident in most places. The extrafascicular connective tissue was in the same structure as the pia mater and it partially surrounded the fascicle in many places. In addition, an intense vascularization was observed in the connective tissue (Figure 2). In

Gordon-Sweet staining, it was observed that reticular fiber bundles started from the connective tissue capsule surrounding the organ and spread by branching. These reticular fiber bundles extended towards the center of the tissue in thin branches (Fig. 3). It was observed that the reticular fiber bundles in the areas where the connective tissue septums expanded were thicker and formed a network (Figure 4). Reticular fibers were also found around blood vessels. In the Well Stain staining method, myelin sheaths were observed around the nerve fibers in the sections after the lamina cribrosa (Figure 5).

It was observed that the pecten oculi started where the intraocularis of nervus opticus (Cr2) ended (Figure 6). It was seen that the intraocularis was sharply thinned as it ended (Figure 7).

Numerous oligodendrocytes with unilocular vacuoles lined up around the fibers were seen in the nerve. Groupings of cells in rows of 3-4 were also observed (Figure 8). In addition, astrocyte extensions formed by oligodendrocytes were seen in transverse and longitudinal sections (Figure 9). Nerve fibers constituted the majority of the visual field of the preparation (Fig. 10). Apart from these, vacuoles were also present in large numbers and in different diameters in Cr2. The ratio of these to the nerve ranged between 20-40% (Figure 11).

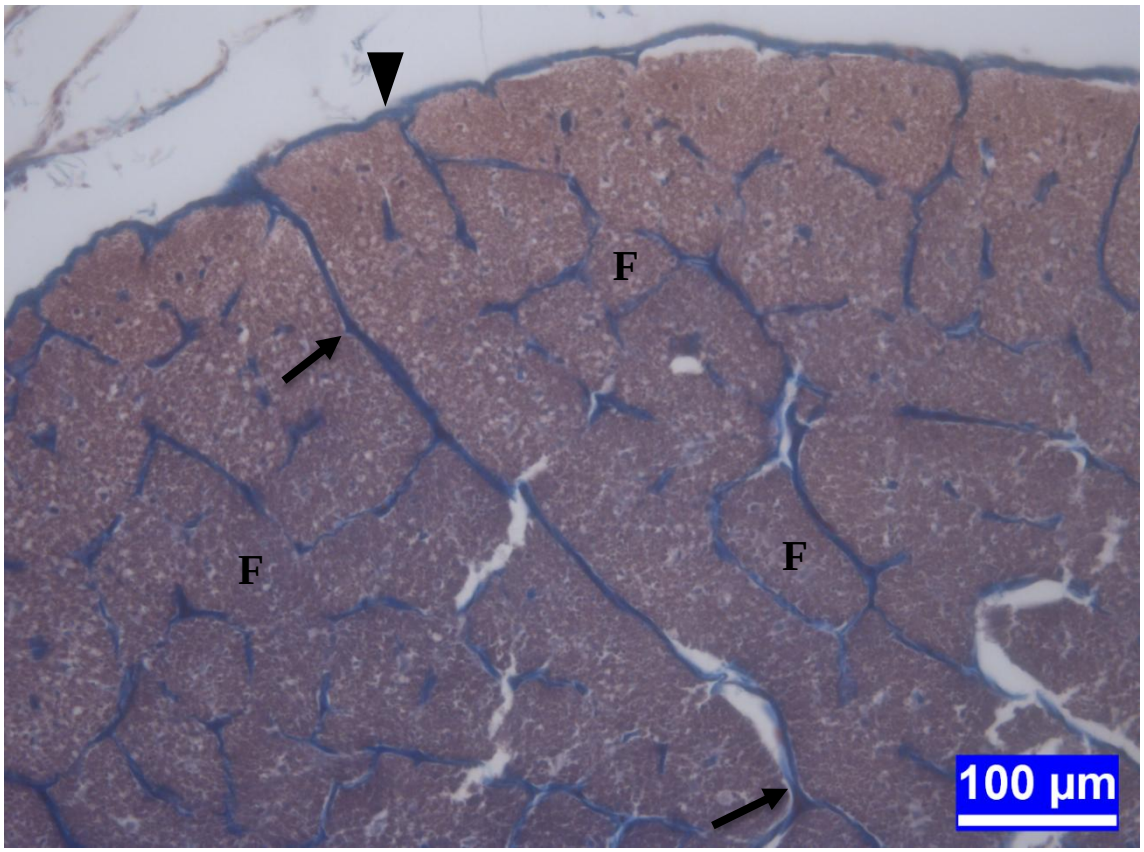

Figure 1. In nervus opticus of chukar partridges (*Alectoris chukar*), the pia mater wraps around the nerve from the outside and sends extensions towards the center of the tissue (arrows). Fascicle (F). Triple staining method

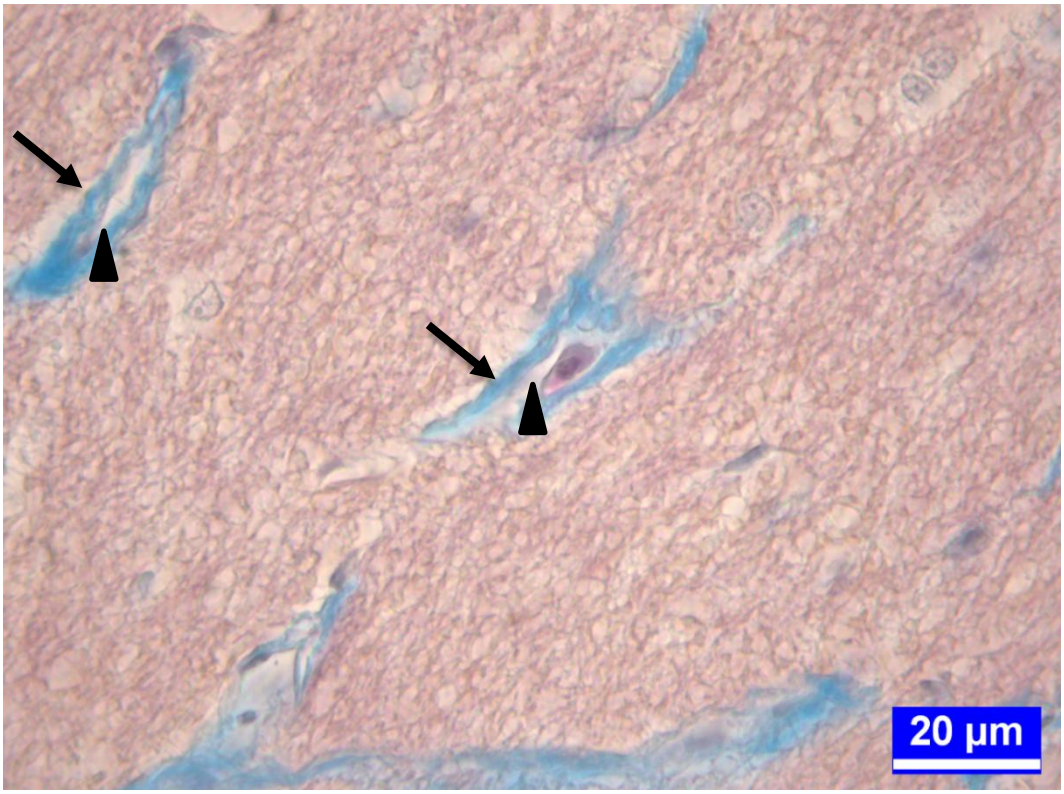

Figure 2. Extrafascicular connective tissue (arrows), blood vessel (arrowhead) in nervus opticus of chukar partridges (*Alectoris chukar*). Triple staining method

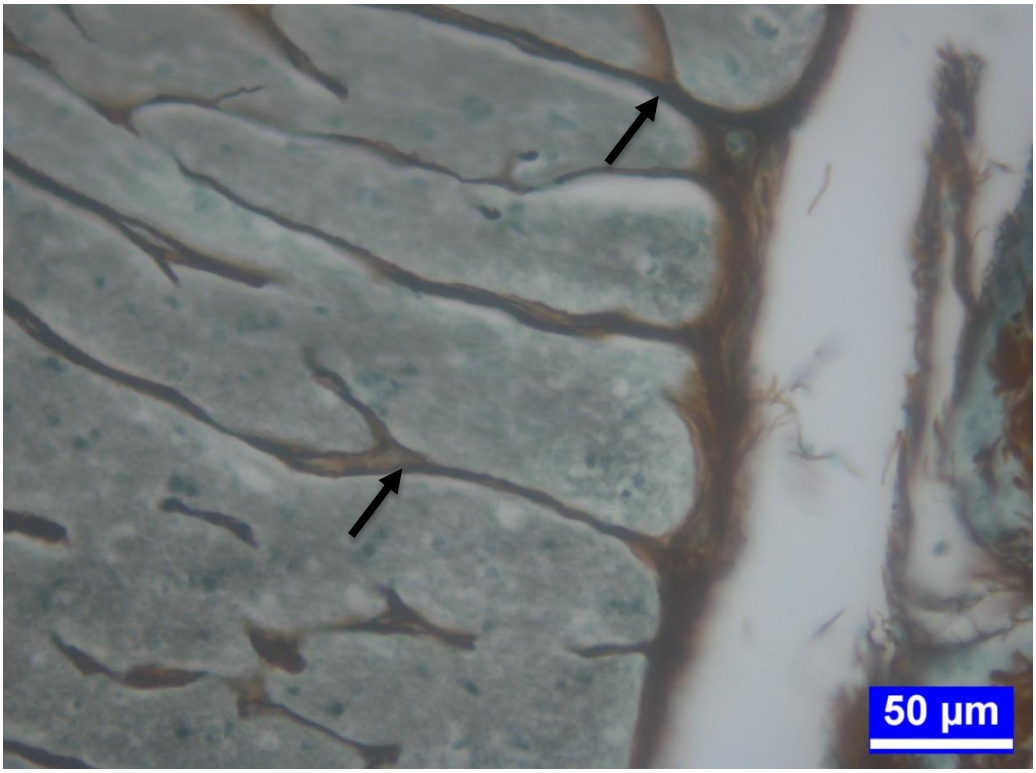

Figure 3. Branching and spreading of reticular fibers in nervus opticus of chukar partridges (*Alectoris chukar*) (arrows). Gordon Sweet (GS) staining method

Figure 4. Reticular fibers (arrow) in areas where connective tissue septums expand in nervus opticus of chukar partridges (*Alectoris chukar*). Gordon Sweet (GS) staining method

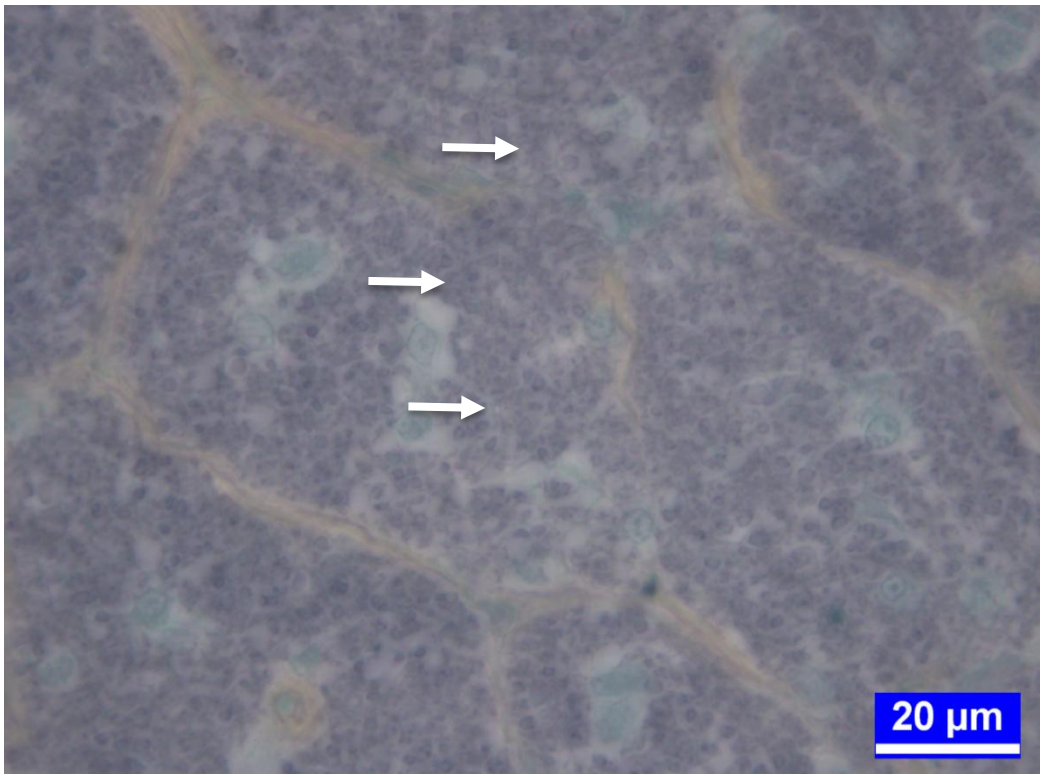

Figure 5. Nerve fibers surrounded by myelin sheath in nervus opticus of chukar partridges (*Alectoris chukar*) (arrows). Well Stain staining method

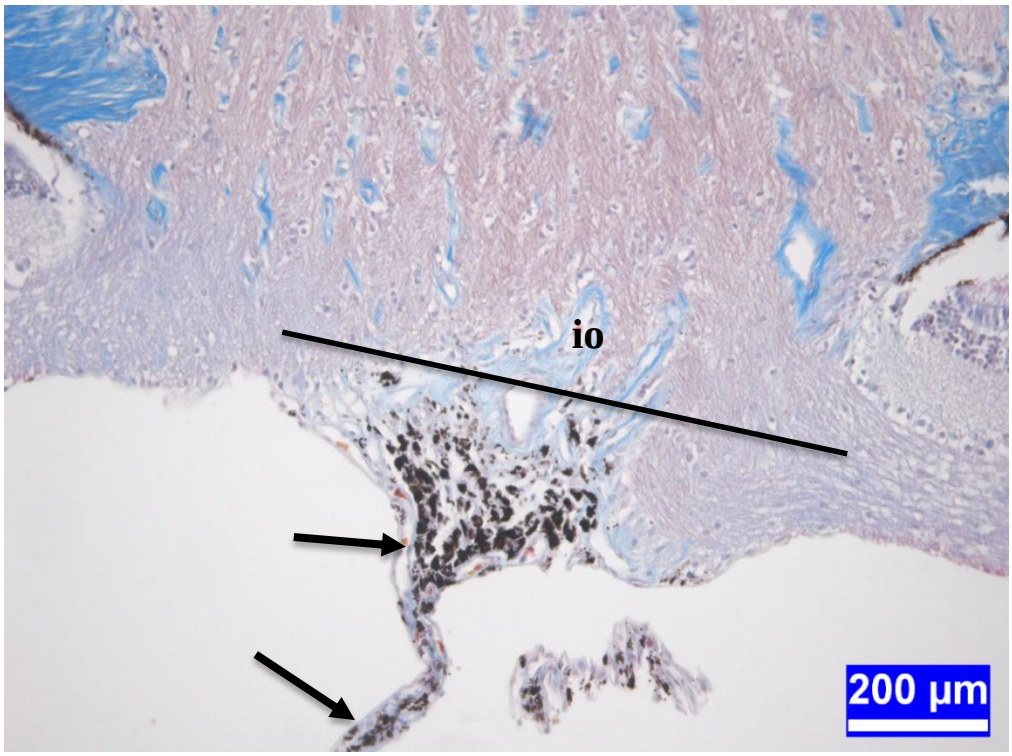

Figure 6. Beginning of pecten oculi (arrows) where intraocularis (io) ends in nervus opticus of chukar partridges (*Alectoris chukar*). Triple staining method

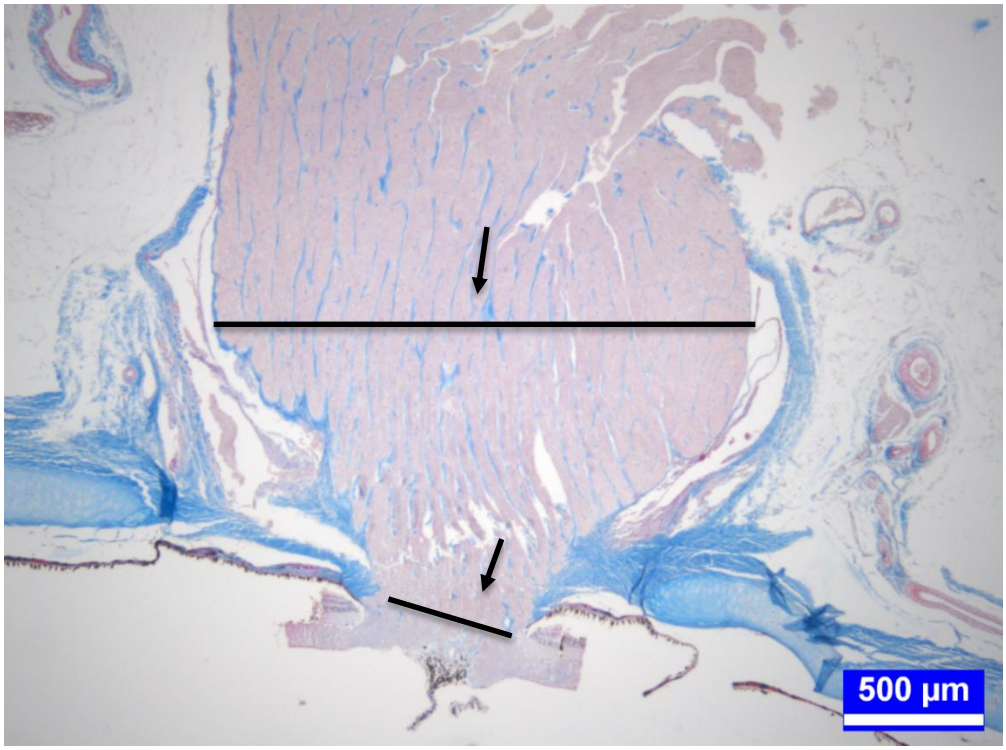

Figure 7. Thinning of the intraocularis as it ends in nervus opticus of chukar partridges (*Alectoris chukar*) (arrows). Triple staining method

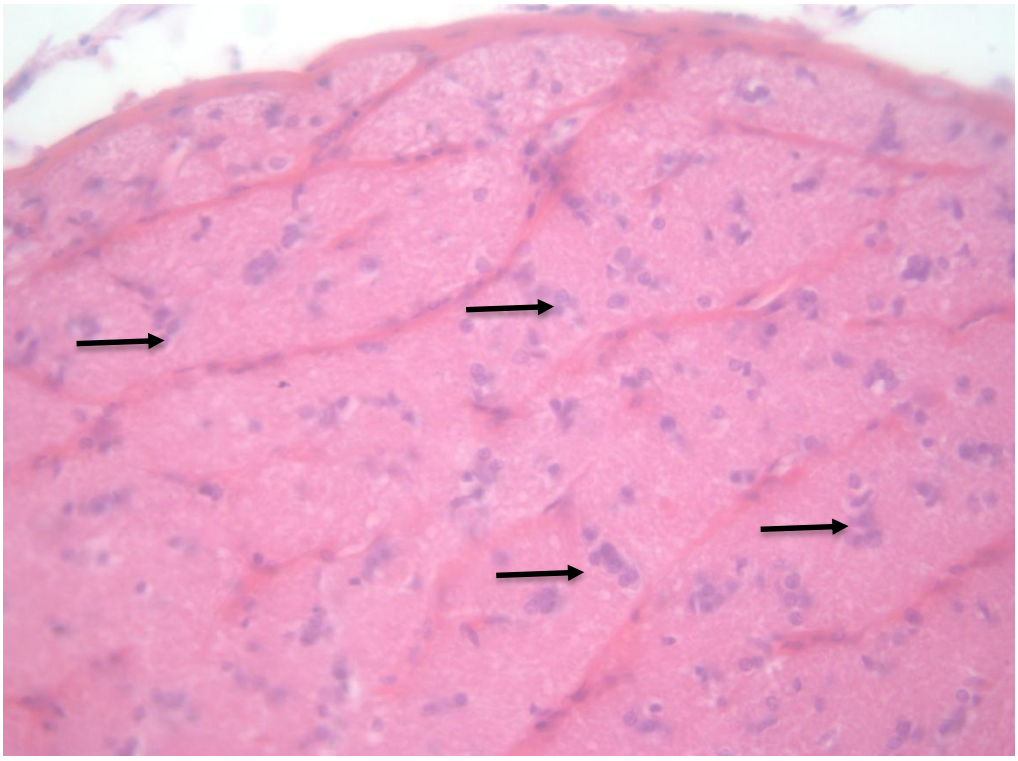

Figure 8. Cell groupings (arrows) in nervus opticus of chukar partridges (*Alectoris chukar*). H&E staining method

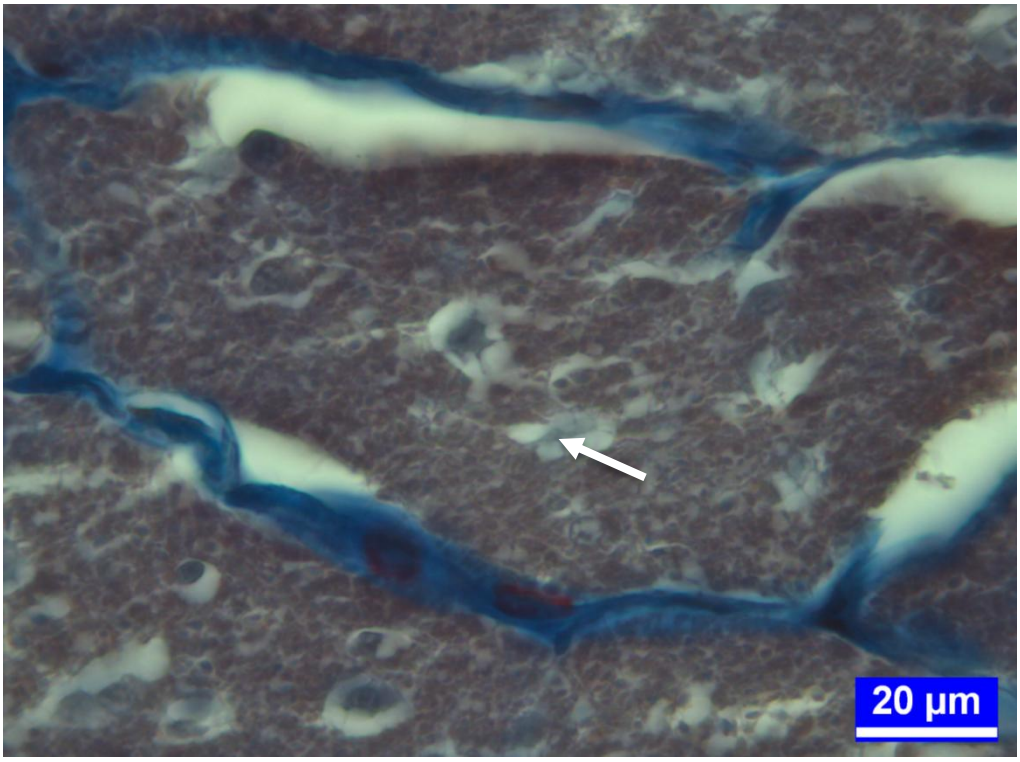

Figure 9. Astrocytes extensions formed by oligodendrocytes in nervus opticus of chukar partridges (*Alectoris chukar*) (arrow). Triple staining method

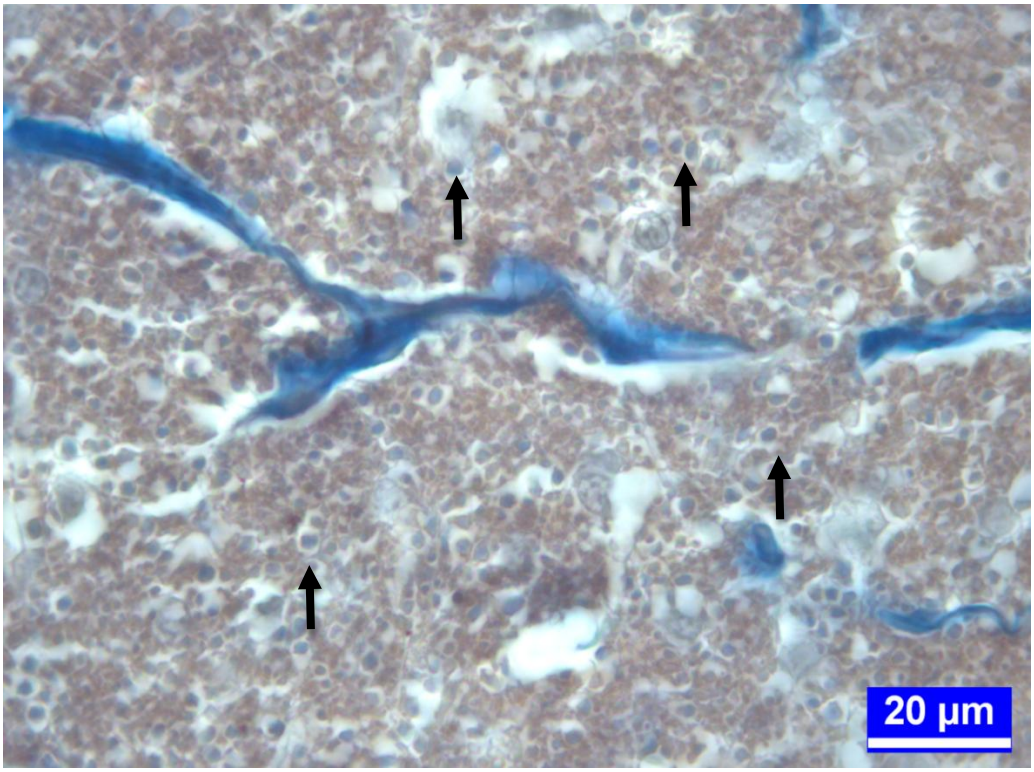

Figure 10. Nerve fibers forming the majority of the visual field in nervus opticus of chukar partridges (*Alectoris chukar*) (arrows). Triple staining method

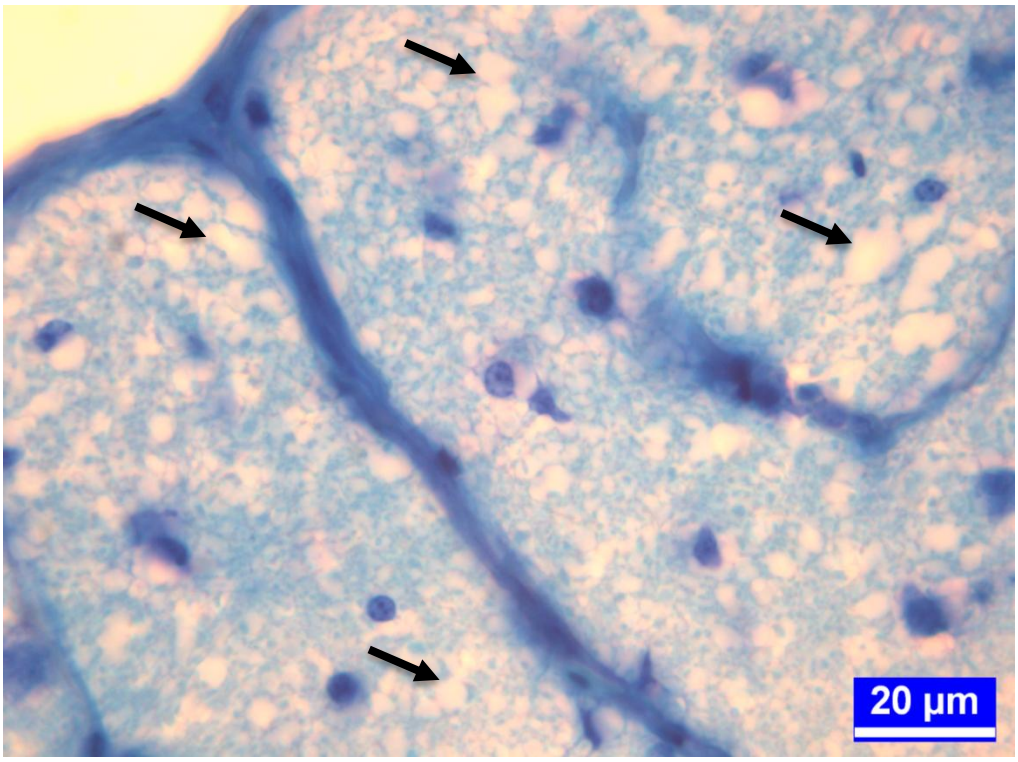

Figure 11. Vacuoles of various diameters in nervus opticus of chukar partridges (*Alectoris chukar*) (arrows). My-Grünwalt Giemsa staining method

4. Discussion And Comment

In general, a nerve fiber consists of axons surrounded by a special sheath derived from cells of ectodermal origin. Nerve fibers differ in their sheaths depending on whether they are located in the peripheral or central nervous system. All the axons of the peripheral nervous system are surrounded by squamous cells called Schwann cells lined up along the extension. Schwann cells form a multilayered membranous wrap around most axons. This wrapping is called myelin, and those wrapped around it are called myelinated fibers. There are no Schwann cells in the central nervous system. Here, glia cells (oligodendrocytes) form the myelin sheath (Soydan, 1992; Junqueira *et al*., 1998). The myelin sheaths of axons in Cr2 are not formed by Schwann cells as in other cranial nerves, but by oligodendrocytes as in the central nervous system (Taner, 2002; Burkitt *et al.*, 1993; Leeson *et al.*, 1985).

Nervus opticus (Cr2) is not a true peripheral nerve, but has developed as an extension of the brain. For this reason, the meningeal structures surrounding the brain are completely surrounded by the nervus opticus (Cr2) from the outside. Duramater, arachnoid and piamater layers are located from the outermost to the innermost, respectively. Piamater and arachnoid are located between dura mater and nerve fascicles. The arachnoid and piamater are directly connected to the nervous tissue. The duramater continues with the sclera where the nervus opticus (Cr2) enters the bulbus oculi, while the piamater and arachnoid membrane continues with the choroid. Meningeal structures include the subdural and subarachnoidal spaces. The subarachnoid space grows near the eye and is called the "vaginal space". The sections of Nervus opticus (Cr2) contain “septas” extending from numerous piamaters and divide the nerve fascicles in polygonal shapes (Krstic, 1991; Kalaycı, 1986; Karaöz, 2002). Nervus opticus (Cr2) fibers are unmyelinated up to the lamina cribrosa (intraocular part), and they are myelinated after the lamina cribrosa. Between the myelinated nerve fibers are glial cells. The number of myelinated fibers of Cr2 in humans is about 1,200,000 on average. Information on the number of myelinated fibers of bird Cr2 is not yet available (Arıcı & Elhan, 1997; Burkitt *et al.*, 1993; Leeson *et al*., 1985; Kalaycı, 1986).

An artery and a vein, which are the vessels of the retina, pass through the center of the nervus opticus (Cr2). The arteria centralis retinae, which progresses towards the bulbus oculi, is separated from the arteria opthalmica, and the vena centralis retinae pours into the arteria opthalmica (Krstic, 1991; Taner, 2002). In our study, we observed a large number of vascularization in the connective tissue located in the center of nervus opticus (Cr2).

Nervus opticus (Cr2) is known as a tract that lies between the ganglion cells in the retina and the diencephalon and actually belongs to the central nervous system. Since it develops from the diencephalon embryologically and is surrounded by meningeal structures throughout its course, it is considered as an extension of the central nervous system, not a peripheral nervous system (Gökmen, 2003; Arıncı, 1994). It is known that the nervus opticus (Cr2) has a cylindrical structure in intraorbital sections (Wagner *et al*., 1997; Arıncı & Elhan, 1993). In our study, the nervus opticus (Cr2) was observed to be cylindrical.

As a result, in our study, nervus opticus (Cr2) of chukar partridge (*Alectoris chukar*) was analyzed histologically and it was aimed to create a resource about avian nervus opticus (Cr2). We believe that our research will also lead the scientists who will do research on this subject. In our study, no atrophic or pathological cell types were found

in the nervus opticus (Cr2). This revealed the necessity of comparing normal and pathological nervus opticus (Cr2) in the avian population in future studies.

References

Anderson, D.R. (1969). Ultrastructure of human and monkey lamina cribrosa and optic nerve head. *Arch Ophthalmol*., 82, 800-814.

Arıcı, K., Elhan, A. (1997). *Anatomy.* Ankara: Güneş.

Arıncı, K. (1994). *Sobotta, Atlas of Human Anatomy*. Istanbul, Beta

Burkitt, H.G., Young, B., Heath, J.W. (1993). *Wheater's Functional Histology*. Longman Group Ltd, Honkong.

Duus, P. (2001). *Nöroloji Tanıda Lokalizasyon*. Ankara, Palma.

Gökmen, G.F. (2003). *Sistematik Anatomi*. İzmir, Guven.

Junqueira, L.C., Carneiro, J., Kelley, R.O. (1998). *Temel Histoloji*. Barış, İstanbul.

Kalaycı, Ş.(1986). *Histoloji*. Uludağ University, Bursa.

Karaöz, E. (2002). *Special Histology.* Suleyman Demirel University, Isparta.

Krstic, R.V. (1991). *Human Microscopic Anatomy*. Springer-Verlag, Berlin Heidelberg.

Leeson, C.R., Leeson, T.S., Paparo, A.A. (1985). *Textbook of Histology*. W.B. Saunders Company, Philadelphia.

Lillie, R.D., Fullmer, H.M. (1965). General staining and mounting procedures. *Histopathologic technic and practical histochemistry, 3rd ed. Blakiston Division, McGraw-Hill, New York,* 84-106.

Mallory, F.B. (1900). A contribution to staining methods: I. A differential stain for connective-tissue fibrillae and reticulum. *J Exp Med*., 5, 15-20.

Oğul, E. (1996). *Temel ve Klinik Nöroloji*. Uludağ University, Bursa.

Radius, R.L. (2001). Anatomy and embriology of the optic nerve. In Kaufmann PL, Mittag T.W., Yanoff M (eds): *Ophthalmology*, Mosby, London, 1994 2.1-2.207. Duus P. Nöroloji Tanıda Lokalizasyon. Palme Yayıncılık, Ankara.

Snell, R.S. (2000). Functional Neuroanatomy for Medical Faculty Students (Translation Editor: Yıldırım M.). Istanbul: Nobel Medical Bookstores and Yüce Publishing.

Soydan, N. (1992). *Genel Histoloji*. Istanbul University, Istanbul.

Taner, D. (2002). *Functional Neuroanatomy*. METU Development Foundation Publishing and Communication Inc., Ankara.

Wagner, A.L., Murtagh, F.R., Hazlett, K.S., & Arrington, J.A. (1997). Measurement of the normal optic chiasm on coronal MR images. *American Journal of Neuroradiology, 18*(4), 723-726.

Williams, P.L., Bannister, L.H., Berry, M.M., Collins, P., Dyson, M., Dussek, J.E., & Ferguson, M.W.J. (1995). The anatomical basis of medicine and surgery. *Gray's Anatomy, 38111*, 512-514.