Age-Related Changes in Rabbit Optic Nerve: A Morphological Study

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Abstract: Aging is associated with neurological symptoms and signs that are suggestive of peripheral neuropathy. The impact of aging on the optic nerve is of general interest. **Objective:** The present work aimed to study the effect of aging on the morphological changes in the optic nerve of rabbit. **Material and methods:** Four groups of Egyptian (Gabali) rabbits (5 rabbits per group) at different postnatal ages; one month old (young), 6 months old (early adult), 18 months old (late adult) and 30 months old (senile) were used in this study. The animals were anaesthetized and rapidly dissected. Optic nerve specimens were obtained and processed for light and electron microscopic (EM) examinations. **Results**: In young animals, the optic nerve fibres appeared as closely packed myelinated axons of small diameters which were separated by processes of astrocytes. In early adult animals, the optic nerve fibres had the same structure to that of the young ones except that the axons were of larger diameters. The astrocytes and oligodendrocytes were observed with no differences in the structure in both young and early adult animals. In late adult animals, the optic nerve fibres were still closely packed but arranged into fascicles by thick processes. There were some depleted areas filled by degenerating axons and neuroglial cells. The degenerating axons showed electron dense axoplasm and redundant sheaths were frequent. The neuroglial cells occupied the depleted areas, were astrocytes and oligodendrocytes. In senile animals, there was an obvious loss of the optic nerve fibres. Extensive degeneration of the nerve axons and their myelin sheath were observed. The astrocytes appeared with pyknotic nucleus and abundant cytoplasmic filaments. The oligodendrocytes had irregular nucleus and the cytoplasm contained vacuoles and inclusion bodies. **Conclusion:** With progress of age, starting from the late adulthood, the optic nerve fibres show degenerative changes in the optic nerve axons, myelin sheath and the neuroglial cells which were to be extensive in senile age.
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Key words: Optic nerve, Histology, Ultrastructure, Aging

1. Introduction

Aging is associated with neurological symptoms and signs that are suggestive of peripheral neuropathy [(1)]. The human longevity increases and brings about an increase in the number of citizens that are elderly. It is needed to understand what is happening to the optic nerve fibres [(2)]. The impact of aging on the optic nerve is of general interest because it appears that aging has global effects on white matter, but relatively little effect on cortical grey matter [(3)]. Elderly humans experience a variety of visual deficits with age, including changes in acuity, accommodation, dark adaptation, visual thresholds, and contrast sensitivity [(4)]. Aging is also associated with an increased incidence of ocular diseases including macular degeneration and glaucoma, as well as systemic disease with ocular complications, such as hypertension and diabetes [(5,6)]. The retinal ganglion cells are unusual elements of the central nervous system because their cell bodies are located in a peripheral organ, the eye, where they have potential exposure to mechanical stresses and environmental factors, such as ultraviolet radiation, that don't affect the rest of the brain. Gradual damage to retinal ganglion cells over the life span due to such factors could be the primary cause of age-related decline in nerve fibre number in the optic nerve [(7-10)]. Although the morphology of the optic nerve has been studied extensively in other species, limited knowledge is available in regard to its morphology in the rabbit. The present work aimed to study whether aging causes detectable changes in the morphology of the optic nerve of rabbit.

2. Material and Methods

Animals:

Four groups of Egyptian (Gabali) rabbits (5 rabbits per group) at different postnatal ages from one month old to senile age were used in this study. The ages were at one month old (young), 6 months old (early adult), 18 months old (late adult) and 30 months old (senile). The animals were obtained from the animal house unit in the Faculty of Veterinary Medicine, Zagazig University, Egypt. The animals were housed in a room of controlled temperature (24.3°C) and were provided *ad libitum* with tap water and fed with standard commercial rabbit chow. The animal procedures were performed in accordance with Guideline for Ethical Conduct in the Care and Use of Animals.

Tissue collection and processing:

The animals were anaesthetized by intra-peritoneal injection of sodium thiopental (4g/L) in a dose of 0.10 ml/10gm body weight. An incision was made in the eyelid so that the eye ball could be gently retracted. The optic nerve was cut behind the globe with microscissor and the eyes were

enucleated and fixed. The optic nerves were dissected free of surrounding tissues. Cross sections of the optic nerve of less than 1 mm in length were taken. The specimens were immediately fixed in 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer at pH 7.4 for 2 hours at 4°C and then washed with the phosphate buffer, post-fixed in 1% osmium tetra-oxide in the same buffer for one hour at 4°C. After washing in phosphate buffer, specimens were dehydrated with ascending grades of ethanol and then were put in propylene oxide for 30 minutes at room temperature, impregnated in a mixture of propylene oxide and resin (1:1) for 24 hours and in a pure resin for another 24 hours. Then, the specimens were embedded in embed 812 resin in BEEM capsules at 60°C for 24 hours [11,12]. Semi-thin sections of about one micron thick were obtained by glass knives and stained with 1% toluidine blue and examined by light microscopy. Ultrathin section of about 50-70 nm thick were cut using diamond knives and mounted on a copper grids, stained with uranyle acetate and lead citrate [11,12] and examined using a JEOL JEM 1010 transmission electron microscope in Electron Microscope Research Laboratory (EMRL) of Histology Department, Faculty of Medicine, Zagazig University.

3. Results

In young animals, the optic nerve axons when examined by light microscopy, appeared closely packed and had small diameters and separated by processes of lightly stained astrocytes. Darker stained cells of oligodendrocytes were observed (Figure 1). By EM examination, all the axons appeared thick myelinated of small diameters which were separated by thick processes of astrocytes. The axoplasm of the nerve fibres appeared electro-lucent and contained microtubules and neurofilaments with mitochondria in between. The astrocytes appeared with pale irregular nucleus and its cytoplasm contained filaments (Figures 2,3). In early adult animals, when examined by light microscopy, the optic nerve fibres had nearly the same morphology except that the axons appeared with larger diameters (Figure 4). By EM examination, the axons appeared with larger diameters and had the same structures to that of the young animals. The oligodendrocytes were characterized by presence of short cisternae of rough endoplasmic reticulum and the astrocytes appeared with characteristic cytoplasmic filaments (Figures 5-7). In late adult animals, the optic nerve fibres when examined by light microscopy were still closely packed but separated into small fascicles by extensive network of astrocytic processes. There were some depleted areas of axons which were occupied by degenerating axons and neuroglial cells (Figure 8). By EM examination, degenerating axons were observed among normal axons. The normal axons appeared myelinated with electro-lucent axoplasm. The degenerating axons appeared with electron dense axoplasm and some were filled with cellular debris. Redundant myelin sheaths were observed (Figure 9). The astrocyte was observed with heterochromatic nucleus and cytoplasm contained inclusion bodies with an increase in the amount of the filaments and many processes extended into the neighbouring axons. The oligodendrocyte was observed with few dilated cisternae of rough endoplasmic reticulum and vacuoles in the cytoplasm (Figures 10,11). In senile animals, the optic nerve fibres when examined by light microscopy, decreased in packing density of the axons with obvious loss of nerve fibres and extensive pale astrocytic processes extending between the axons and separating them into bundles. Many neuroglial cells were observed in between (Figure 12). By EM examination, the axons were observed thin myelinated which were separated by thick astrocytic processes. The myelin sheaths showed splitting of their myelin lamellae and contained dark cytoplasm; and some accommodated fluid-filled balloons. Some axons appeared losing their sheaths and most of the axons appeared degenerated with electron dense axoplasm. In some axons, the neurofilaments were disrupted with enlargement of axoplasmic spaces and even some axoplasms were devoid of neurofilaments. Some myelin sheaths appeared redundant and others appeared thin and ballooned out (Figures 13-16). The astrocyte was observed with pyknotic nucleus and abundant amount of cytoplasmic filaments (Figure 15). The oligodendrocyte was observed with irregular nucleus and the cytoplasm contained vacuoles and inclusion bodies (Figure 16).

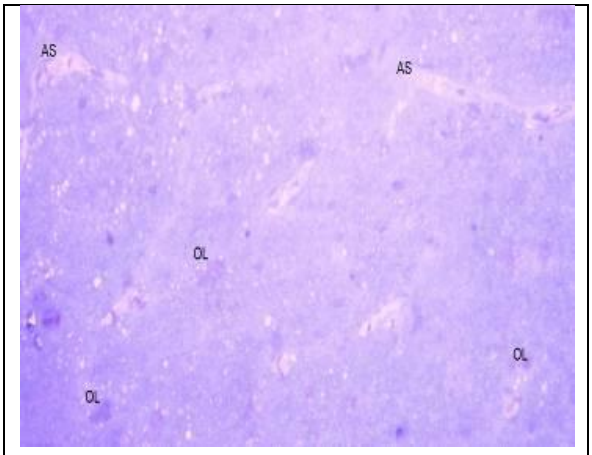

Figure (1): A photomicrograph of a semi-thin section in the optic nerve of young rabbit showing closely packed axons of small diameters and separated by processes of lightly stained astrocytes (AS). Darker cell bodies of oligodendrocytes (OL) are seen. (Toluidine blue X 400)

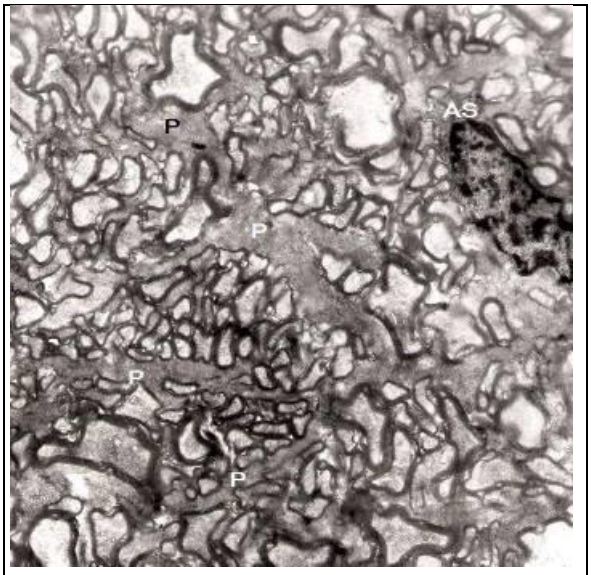

Figure (2): An electron photomicrograph of ultrathin section in the optic nerve of young rabbit showing closely packed myelinated axons of small diameters. The axons are separated by thick processes (p) of astrocytes (AS). (X 9000)

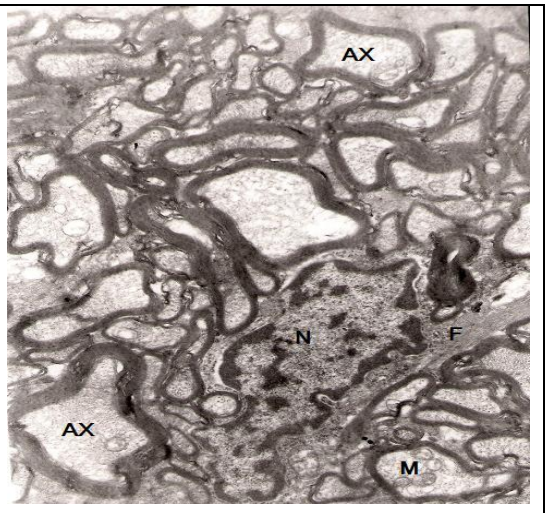

Figure (3): An electron photomicrograph of ultrathin section at a higher magnification in the optic nerve of young rabbit showing thick myelinated nerve axons. The axoplasm (AX) of the nerve fibres is electro-lucent and contains microtubules and neurofilaments with mitochondria (M) in between. An astrocyte appears with pale irregular nucleus (N) and the cytoplasm contains filaments (F). (X 16000)

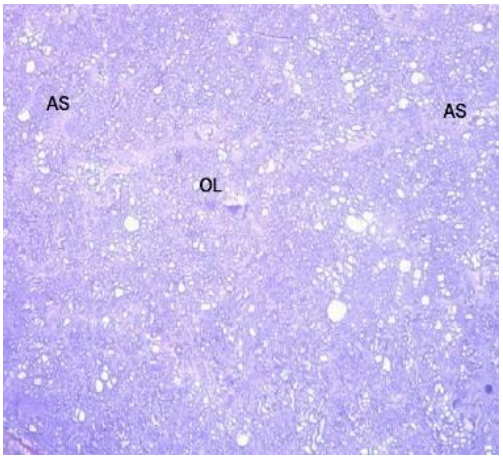

Figure (4): A photomicrograph of a semi-thin section in the optic nerve of early adult rabbit showing closely packed nerve axons of larger diameters. Lightly stained astrocytes (AS) appear with their processes extending between the axons. Darker stained oligodendrocytes (OL) are seen. (Toluidine blue X 400)

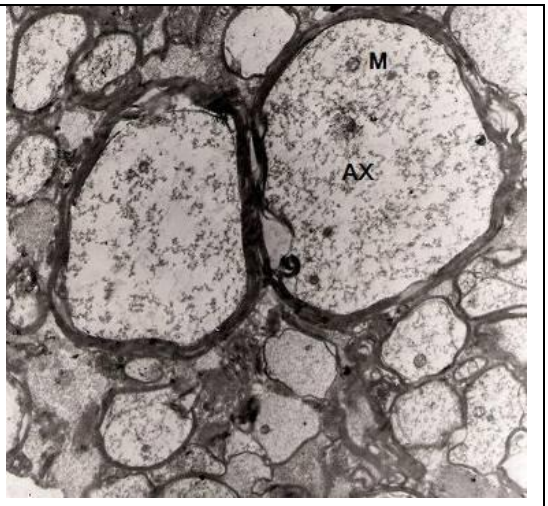

Figure (5): An electron photomicrograph of ultrathin section in the optic nerve of early adult rabbit showing myelinated nerve axons of larger diameter. The axoplasm (AX) is electro-lucent and contains microtubules and neurofilaments with mitochondria (M) in between. (X 9000)

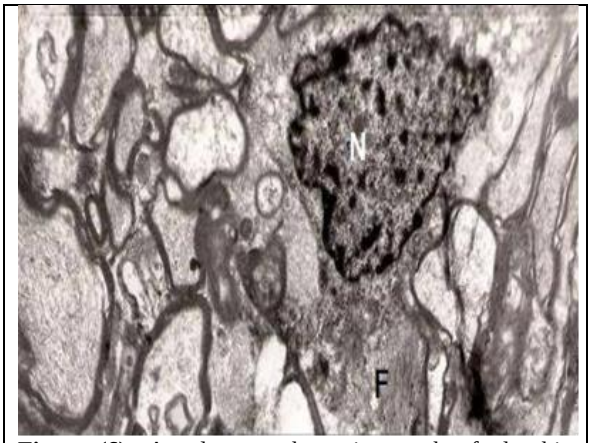

Figure (6): An electron photomicrograph of ultrathin section in the optic nerve of early adult rabbit showing the astrocyte. The nucleus (N) is irregular and the cytoplasm contains filaments (F). (X 9000)

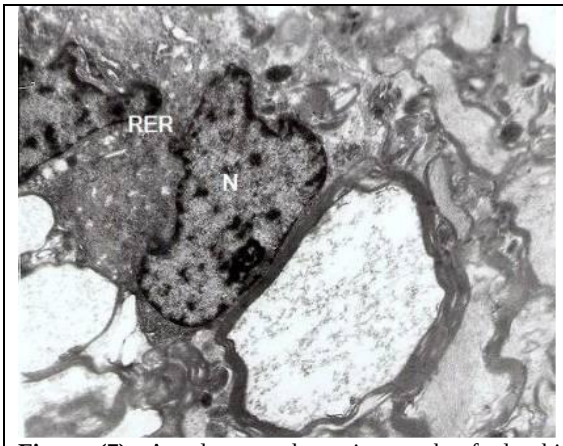

Figure (7): An electron photomicrograph of ultrathin section in the optic nerve of early adult rabbit showing the oligodendrocyte. The cytoplasm is characterized by presence of short cisternae of rough endoplasmic reticulum (RER) near the nucleus (N). (X 9000)

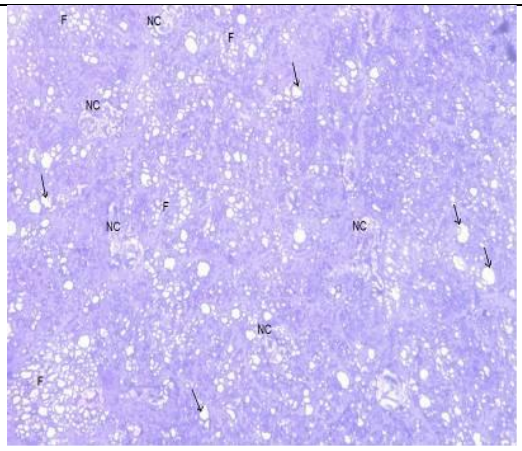

Figure (8): A photomicrograph of a semi-thin section in the optic nerve of late adult rabbit showing that, in some areas, the axons are still closely packed but separated into small fascicles (F) by extensive network of astrocytic processes. The other areas are depleted and occupied by degenerating axons (arrow) and neuroglial cells (NC). (Toluidine blue X 400)

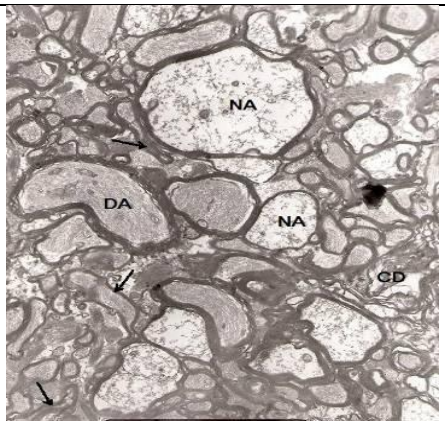

Figure (9): An electron photomicrograph of ultrathin section in the optic nerve of late adult rabbit showing degenerating axons (DA) among normal axons (NA). The normal axons appear myelinated with pale axoplasm. The degenerating axons have electron dense axoplasm or the axoplasm is filled with cellular debris (CD). Redundant sheaths are seen (arrows). (X 9000)

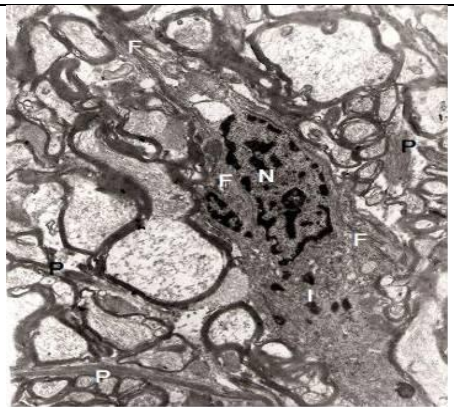

Figure (10): An electron photomicrograph of ultrathin section in the optic nerve of late adult rabbit showing the astrocyte. The nucleus (N) appears heterochromatic with chromatin bodies. The cytoplasm shows abundant filaments (F) and inclusion bodies (I). Many processes (P) extend to the neighbouring axons. (X 9000)

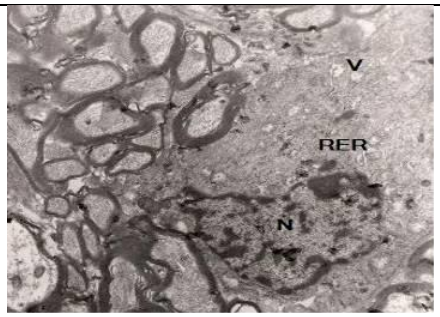

Figure (11): An electron photomicrograph of ultrathin section in the optic nerve of late adult rabbit showing the oligodendrocyte. The cytoplasm shows few dilated cisternae of rough endoplasmic reticulum (RER) near to the nucleus (N) and vacuoles (V). (X 9000)

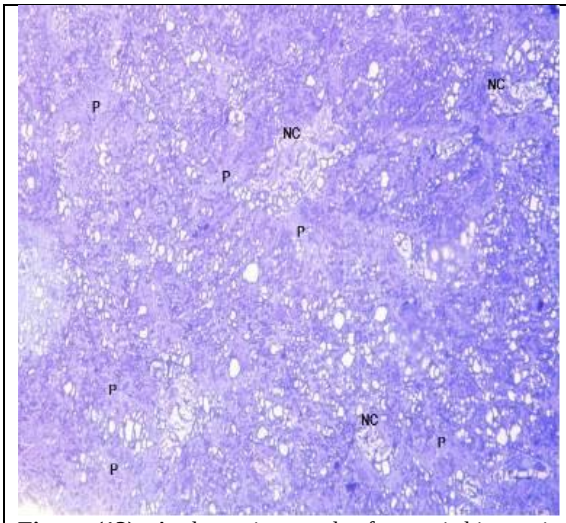

Figure (12): A photomicrograph of a semi-thin section in the optic nerve of senile rabbit showing a decrease in the packing density of the axons and obvious loss of nerve fibres. Extensive astrocytic processes (P) extend between the axons and separate them into bundles and many neuroglial cells (NC) are seen in between. (Toluidine blue X 400)

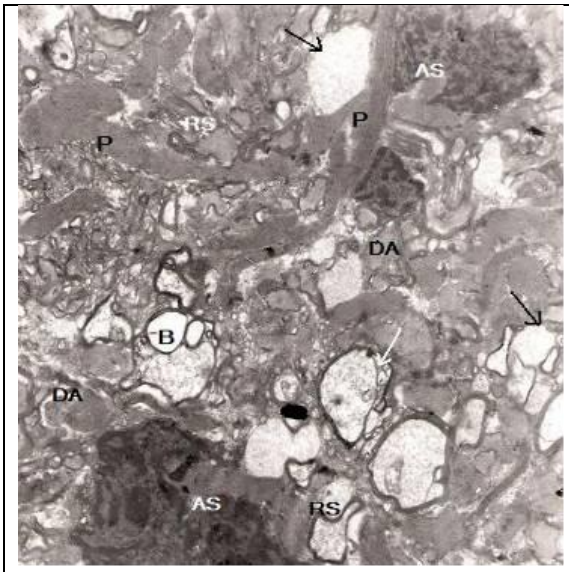

Figure (13): An electron photomicrograph of ultrathin section in the optic nerve of senile rabbit showing thin myelinated axons which are separated by thick astrocytic processes (P). The myelin sheath shows splitting of myelin lamellae and accommodate dark cytoplasm (white arrow) and some accommodate fluid-filled balloons (B). Some axons appear losing their sheaths (dark arrows). Most of the axons appear degenerated (DA) with electron dense axoplasm. Astrocytes (AS) and redundant sheaths (RS) are seen. (X 9000)

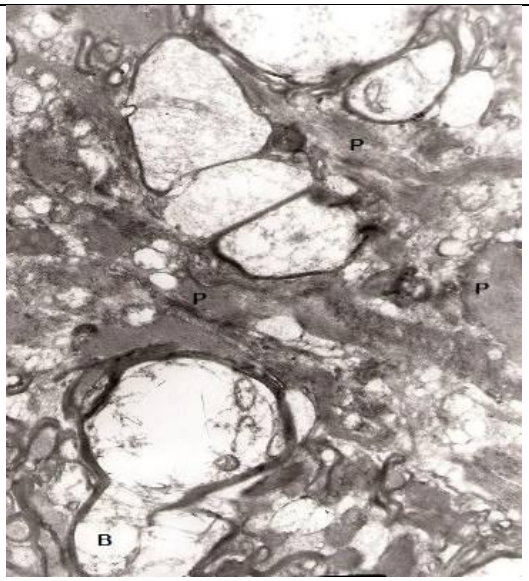

Figure (14): An electron photomicrograph of ultrathin section in the optic nerve of senile rabbit showing degenerating axons with disruption of the neurofilaments. The axons are separated by thick processes of connective tissue (p). Some axons appear empty and others appear with electron dense axoplasm. The myelin sheath appears thin and ballooned out (B). (X 15000)

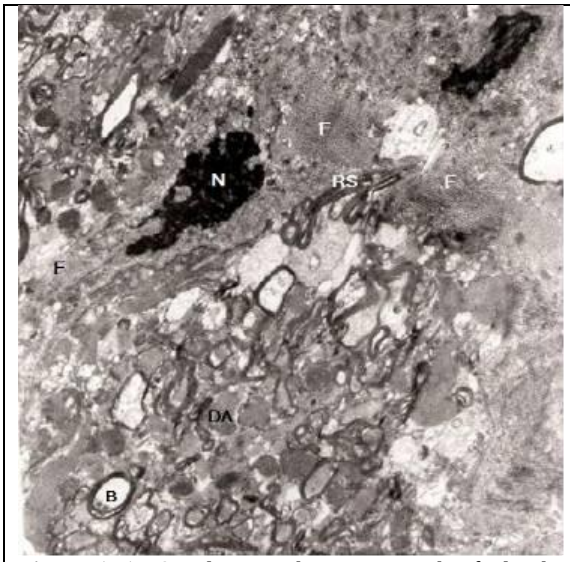

Figure (15): An electron photomicrograph of ultrathin section in the optic nerve of senile rabbit showing the astrocyte with pyknotic nucleus (N) and the cytoplasm contains abundant amount of filaments (F). The axons surrounding the cell are degenerated with electron dense axoplasm (DA). The myelin sheaths are thin and lost in many axons and some are redundant (RS). Fluid-filled balloons (B) are seen. (X 9000)

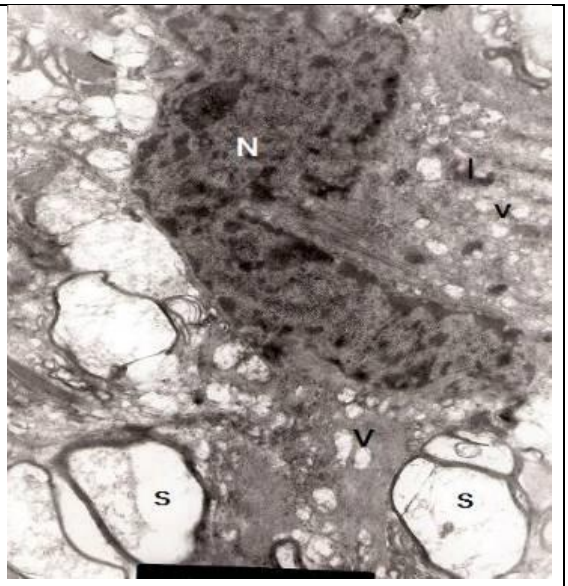

Figure (16): An electron photomicrograph of ultrathin section in the optic nerve of senile rabbit showing the oligodendrocyte. The nucleus (N) appears irregular. The cytoplasm contains vacuoles (V) and inclusion bodies (I). The axons appear degenerated with disruption of the neurofilaments and enlargement of axoplasmic spaces (S). (X 15000)

4. Discussion:

In the present study, the optic nerve fibres in young rabbits were observed as myelinated axons of small diameters which were separated by processes of astrocytes. The axoplasm appeared electro-lucent and formed of microtubules and neurofilaments with mitochondria in between. In early adult animals, the optic nerve fibres had the same structure except that, the axons were of larger diameters. This is in agreement with Harris *et al.* **(13)** who observed that, by postnatal day 30 the majority of axons were myelinated. Hunter and Bedi **(14)** found that, the myelinated optic nerve fibres are first observed at about postnatal day 5-7 with about 90% being myelinated by 25 day of age. Also, Crespo *et al.* **(15)** and Kuwabara **(16)** found that, by the end of the 3rd postnatal week the rat optic nerve is developed into its adult form. As regards to the neuroglial cells observed in the present study, it was found that, the astrocytes and oligodendrocytes were observed in the optic nerve of young and early adult rabbits with the same structure in both ages. The astrocyte appeared lightly stained and had the characteristic cytoplasmic filaments and the oligodendrocytes appeared darkly stained with few short dilated cisternae of rough endoplasmic reticulum. This is in consonance with Zhang and Mckanna **(17)** who found that, the number of astrocytes at birth is very similar to that in the adult. Skoff **(18)** and Skoff *et al.* **(19)** found that, the oligodendrocytes are generated after birth and most of them are generated within the first 3-4 postnatal weeks. Peters **(20)** mentioned that, in young monkeys, the oligodendrocytes are recognized by having a dark nucleus which is surrounded by an electron-dense cytoplasm with short and dilated cisternae of rough endoplasmic reticulum, polyribosomes, mitochondria. The astrocyte has pale nucleus with very little clumping of chromatin. The unique component in the cytoplasm of astrocytes is the intermediate filaments which aggregate into bundles that pass into the processes. In the present study, the optic nerve fibres in late adult rabbits were still closely packed but arranged into fascicles by thick processes. Some depleted areas were filled by degenerating axons and neuroglial cells. The degenerating axons appeared among normal axons and showed electron dense axoplasm and some were filled with cellular debris. Redundant sheaths were frequent. This is in agreement with Cepurna *et al.* **(21)** who found that, a low ratio of degenerating axons to total axons in the 5-months group increased exponentially with age. Lampert **(22)** found that, the evidence of degeneration of the axons of myelinated nerve fibres is indicated by the appearance of axonal profiles with darkened axoplasm. Bowley *et al.* **(23)** added that such degenerating axons are found in both middle aged and elderly monkeys and often contain vacuoles and dense debris. Peters **(24)** mentioned that, the presence of dense cytoplasm within the sheath is an indication that it is degenerating. Also, Sturrock **(25)** found that, there are two types of abnormality were seen in the myelinated fibres at all ages after 12 months in rats. These consisted of degenerating axons enclosed in normal myelin sheaths and apparently normal axons surrounded by vacuolated sheaths. Moreover, there is a continuous loss and replacement of myelinated axons in both limbs of anterior commissure from 12-31 months of age. In the present study, the redundant sheaths were observed in late adult animals. This is in accordance with Bowely *et al.* **(23)** who found that, the frequency of redundant myelin sheaths predominates in middle age than in old age. On the other hand, Peters **(20)** found that, redundant sheaths can be found even in young monkeys, although the frequency of their occurrence increases with age. Peters **(24)** and Peters *et al.* **(26)** related the appearance of the redundant myelin sheaths to the continued formation of myelin with age. In the present study, there was an increase in the frequency of the neuroglial cells that occupied the depleted area of normal axons. These neuroglial cells were astrocytes and oligodendrocytes. The astrocyte was characterized by the presence of dark inclusion bodies and increased cytoplasmic filaments. The oligodendrocyte was characterized by the presence of few short dilated rough endoplasmic reticulum, dark inclusion and vacuoles. This is in agreement with Sandell and Peters **(27)** who found that, the neuroglial cells

frequently have inclusions in their cytoplasm. This increase in the frequency of neuroglial cells is most obvious in the optic nerves that show extensive degeneration of nerve fibres. Also, peters and Sethares [28] found that, with age, the oligodendrocytes accumulate increasing numbers of dense inclusions in their perikarya. The dense material is related to the activity of the cell. Moreover, with increasing age, the oligodendrocytes are preferentially generated, aiding to remyelinate the axons following demyelination [29,30]. Cavallotti *et al.* [31] mentioned that, the age related changes in rat optic nerve show increased number of astrocytes. On the other hand, Sandell and Peters [32] mentioned that, during normal aging, the astrocytes don't increase with age and added that, the astrocytes develop abundant filaments and undergo hypertrophy to fill spaces vacated by the degenerating nerve fibres. In senile animals of the present study, there was obvious loss of optic nerve fibres which were separated by thick processes and filled with neuroglial cells. Extensive degeneration of the nerve axons and their myelin sheaths were observed. The myelin sheath appeared thin with splitting of myelin lamellae to accommodate dark cytoplasm or fluid filled balloon. Some myelin sheaths appeared redundant and others appeared ballooned out. The axons appeared degenerated with electron dense cytoplasm. The neurofilaments were disrupted with enlargement of axoplasmic spaces. This is in agreement with Sandell and Peters [27] who found that, the dominant change in the optic nerves affected by age in all old monkeys is the degeneration of axons. This axonal degeneration ultimately is followed by its phagocytosis by neuroglial cells. The spaces in the optic nerve occupied by the fibrous trabeculae increase with age. They added that, the most common myelin abnormalities in the aging optic nerve in monkeys are splitting of the major dense line to accommodate dense cytoplasm and ballooning of a myelin sheath. Bowley *et al.* [23] and Peters [24] added that, the dense cytoplasm may be inclusion, lysosomes and vacuoles and their presence within the sheaths is an indication of degeneration. Also, it is suggested that, the ballooning of myelin sheath is a degenerative change since similar balloons can be produced experimentally such as ballooning of myelin occurs in severe diabetes [33] and in early phases of Wallerian degeneration in the dorsal funiculus of the spinal cord when myelin breaks down as a consequence of dorsal rhizotomy [34]. Peters et al. [35] found that, these age-related myelin changes are identical to these observed in other parts of the aging brain. Myelin balloons arise from splitting of the myelin sheath at the intraperiod line [23]. These balloons appear as spherical bulge of the sheaths that are filled with fluid [36]. The redundant sheaths observed in senile rabbits of the present study are in accordance with Peters [20] who found an increase in the frequency of redundant myelin in the nerve fibres with aging. In a study of the effects of age on myelin sheath in the white matter of mice, Sturrock [37] found such redundant sheaths to be common in old mice. Rosenbluth [38] suggested that, these irregular myelin may result from the active formation of myelin. The thin myelin observed in the present study in senile rabbits is supported by Bowley *et al.* [23] who found that, in old monkeys, some fibres have inappropriately thin myelin sheaths composed of only two or three layers of myelin. Ludwin [39] and Hirano [40] mentioned that, such thin myelin sheaths are generally regarded as an indicator for remyelination in CNS. Other studies indicated that, the overall capacity of the old brain to remyelinate may be limited. A decreased capacity for remyelination have been found following experimentally induced demyelination [41,42]. Spontaneous remyelination of demyelinated lesions preferentially involves axons that are less than 1 µm in diameter [43]. In cats, Xun *et al.* [44] observed that, the axons of the optic nerve fibres in old cats were swelled accompanied with loosened sheath, structural disorder, lamella vacuolization and even sheath collapse. Malone and Szoke [45] suggested that, these changes may cause an increased fluidity and decreased stability of myelin. Sloane *et al.* [46] also found the composition of myelin to alter with age, suggesting that there is new formation of myelin by oligodendrocyte, perhaps in response to myelin degeneration and injury caused by proteolytic enzymes such as calpain, which increases in white matter with age. On the contrary, some authors reported thick myelin with aging, Godlewski [47] found that the myelin sheaths in the corpus callosum and optic nerves of 2.5 years old rats were thicker than those of 4 months old rats. Also Caselli *et al.* [48] found no changes in the number of lamellae in the sciatic nerve of old rats while Ceballos *et al.* [49] reported that in the tibial nerve of mice, myelin sheaths become thicker between 6 and 33 months of age with some sheaths becoming very thick. The astrocytes in the optic nerve of senile rabbits in the present study, were observed with pyknotic nucleus and abundant amount of cytoplasmic filaments. The oligodendrocytes were observed with irregular nucleus and the cytoplasm contained vacuoles and inclusion bodies. This is in agreement with Sandell and Peters [32] who found that, with aging the astrocytes don't increase in number but they develop abundant filaments and the oligodendrocytes have dark inclusions. On the other hand Xun *et al.* [50] found that, in old cats the astrocytes appeared hypertrophied and occupied larger proportion among total glial cells when compared with that in young adult ones. Peters *et al.* [51] and Peters [52] found that, in old monkeys, the oligodendrocytes have a difference in that,

many of them have dense inclusions in their perikaryal cytoplasm. These dense inclusions are irregular in shape. Also, Vaughan and Peters [53] reported the existence of similar inclusion in the oligodendrocyte, within the auditory cortex of aging rats and Rees [54] reported dense inclusions in oligodendrocytes in the cerebral cortex of aged human brains. Levine and Torres [55] suggested that, the inclusions might be derived from the degeneration of some component of the aging myelin sheath attached to the oligodendrocytes. Levine *et al.* [56] found that, in aging, additional oligodendrocytes are required to remyelinate the axons after their degeneration and that cells are derived from oligodendroglial progenitor cells that are present in the mature CNS. On the other hand Cerghet *et al.* [57] found that, in aging rats, some oligodendrocytes undergo apoptosis with age, suggesting that degenerative myelin sheath alternations in aging may result from death of the parent oligodendrocyte.

It could be concluded that, with progress of age, starting from the late adulthood, the optic nerve fibres had degenerative morphological changes in the nerve axons, myelin sheath and the neuroglial cells which increased to be extensive degenerative changes in senile age. This conclusion is in general consistent with clinical studies, showing that the degeneration varies from one person to another and in some instances it may be great enough to produce marked deficits [58,59].

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