CPQ Neurology and Psychology (2019) 1:5
Research Article

# Severe Traumatic Brain Injuries in Aging Patients. A Light and Electron Microscopic Study

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Received: 25 April 2019

Published: 03 June 2019

**Keywords:** *Brain Trauma, Brain Aging, Neurons, Glial Cells, Microscopy*

## Abstract

We have studied the pathological changes involved on severe and complicated human head traumas associated to subdural hygroma or hematoma in old patients. Light and electron microscopy study of four patients ranging from 58 to 80 years old, with severe brain traumas and associated subdural hygroma and hematoma were examined. Light microscope showed status spongiosus of brain parenchyma, apoptotic, oncotic, autophagic and necrotic nerve cell death types, reactive hypertrophic astrocytes containing lipofucsin granules, dense oligodendrocytes, and degenerated myelinated axons. Electron microscopy of edematous nerve cell cytoplasm exhibited vacuolar enlargement of rough endoplasmic reticulum with detachment of associated ribosomes, nucleus with distended nuclear pores and irregularly dilated nuclear envelope, swollen mitochondria, enlarged extracellular space, degenerated myelinated axons, and synaptic plasticity and degeneration. Satellite perineuronal, interfascicular and perivascular glycogen depleted-and glycogen-rich astrocytes. Astrocytes showed clustered and thin vesicular profiles of smooth endoplasmic reticulum. Lipofucsin-rich astrocytes also were found. The degenerated myelinated axons appeared attached to swollen and ischemic oligodendrocytes. Close examination of the neuropile showed synaptic plasticity and degeneration.

Blood brain barrier breakdown was observed featured by increased surface activity of luminal endothelial cell surface and vesicular and vacuolar transendothelial transport, basement thickening, swollen pericites with dense bodies, and swollen astrocyte end-feet anchored to the outer surface of basement membrane. Multilayered basement membrane separated by thin bands of swollen pericite cytoplasm was found bearing proliferation of collagen fibers. Perivascular dense microglial cells also were also seen. The findings indicate cytotoxic and vasogenic brain oedema, cell death, and neurodegenerative processes leading to neurological and mental diseases.

## Introduction

Traumatic brain injury (TBI) has come to the forefront of the scientific investigation at the present time. [1] reported specific impairment in attentional control processes, exhibited as a deficit in focused attention. Head trauma is considered to be a risk factor for Alzheimer's disease, because a high prevalence of beta AP deposits has repeatedly been reported in patients who died within a few days following head injury, The density of beta AP diffuse deposits was linked only to aging and the presence of senile plaques [2]. Dobrokhotova *et al.* [3] (1996) described that confusion was common in adults elderly and old patients. According to Bigler *et al.* [4] (2002) age-related changes cause minimal temporal lobe gyral, hippocampal, temporal horn, and white matter atrophy. Only subarachnoid sulcal CSF volume changed robustly brain trauma produced disproportionate White matter loss associated with increased temporal horn and sulcal CSF volumes; it caused substantial hippocampal atrophy, which was related to memory impairment. Gyral volume did not decrease, although it was related to memory performance. Miakotnykh *et al.* [5] (2007) found predominance of vessels neurological symptoms in combination with cranial-cerebral trauma and ischemic stroke. Some symptoms of cranial-cerebral trauma predominate only in acute traumatic period. But the vessels neurological symptoms become prevailing again after successful treatment in connection with cranial-cerebral trauma. This determines that cerebral vessels pathology always predominates as regards cerebral traumatic pathology in old patients.

Hanif *et al.* [6] (2009) examined the age related outcome in acute subdural haematoma following traumatic head injury and concluded that the elderly are most predisposed to bleeding due to normal cerebral atrophy related to aging, stretching the bridging veins from the dura. Sayed *et al.* [7] (2013) found that patients with dementia after TBI were significantly more likely to experience depression, anxiety, irritability, and motor disorders than patients with probable Alzheimer disease.

Norden *et al.* [8] (2015) emphasize the microglial priming and enhanced reactivity to secondary insult in aging, and traumatic CNS injury, and neurodegenerative disease, and that microglia can develop a primed or pro-inflammatory mRNA, protein and morphological profile with aging, traumatic brain injury and neurodegenerative disease.

The pathophysiological processes involved on severe head traumas in elderly patients, and the individual traits that influence susceptibility to neurocognitive diseases has been recently studied [9]. Some clinical studies has reported a relationship between traumatic brain injury (TBI) and neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases, as well as certain psychiatric diseases [10,11].

Memory is fundamental to everyday life, and cognitive impairments resulting from traumatic brain injury (TBI) have devastating effects on TBI survivors. A contributing component to memory impairments caused by TBI is alteration in the neural circuits associated with memory function [12].

There is evidence that human and rodent microglia may become senescent. This event determines alterations in the microglia activation status, associated with a chronic inflammation phenotype and with the loss of neuroprotective functions that lead to a greater susceptibility to the neurodegenerative diseases of aging [13].

Recent research with neuropathologic or biomarker evidence of Alzheimer's disease (AD) casts doubt on traumatic brain injury (TBI) as a risk factor for AD. Research that evaluates number and severity of TBIs is needed to clarify the neuropathological links between TBI and dementia documented in other large clinical databases [14].

Electron microscopy studies of human severe TBI and aging brain offer the possibility to increase our understanding of brain aging mechanisms, neuroinflammation, and neurodegeneration following TBI. The present study is an attempt to a deeper insight on the pathomorphology of severe and complicated trauma and brain aging, specifically studying living cortical biopsies taking during neurosurgical treatment of patients ranging from 57 to 80 years old, with severe and complicated brain injuries.

## Material and Methods

Samples of cerebral cortex of four patients with clinical diagnosis of severe traumatic brain injuries associated to subdural hygroma or haematoma were used in the present study. Cortical biopsies were performed according to basic principles of the Declaration of Hel¬sinki and Ethical Committee of Biological Research Institute. Parent consent was obtained in each case. Table 1 contains the clinical data and lists the cortical regions from which the biopsy were taken. Two to five mm thick cortical biopsies were immediately fixed in the surgical room in 4% glutaraldehyde-0.1 M phosphate or cacodylate buffer, pH 7.4 at 4˚ C. Later they were divided into 1 mm fragments and immersed in a fresh, similar solution for periods varying from 2 to 72 h, followed by secondary fixation in 1% osmium tetroxide-0.1 M phosphate buffer, pH 7.4 for 1 h. They were then rinsed for 5 to 10 min in a buffer similar to that used in the fixative solution, dehydrated in increasing concentrations of ethanol and embedded in Araldite or Epon. For proper orientation of the electron microscope study, thick sections of approximately 0.1 to 1μm were stained with toluidine blue and examined with a Zeiss photomicroscope. Ultrathin sections obtained with a Porter-Blum and LKB ultramicrotomes were stained with uranyl acetate and lead citrate and ob¬served in a JEOL 100B electron microscope. Observations were made using magnification from X 720 to X 840 magnifications from light microscope, and intermediate magnifications from transmission electron microscope ranging from 60.000 to 75.000 X. The cortical biopsies were taken from the perifocal edematous areas of brain parenchyma, and immediately included in gluraraldehyde buffer solutions for avoiding delayed fixation artefacts and post-mortem changes.

## Neurosurgical and Clinical Study

| Sample Identification | Age and Sex | Clinical Data | Diagnosis | Cortical Biopsy |
|---|---|---|---|---|
| *Case 1* | | | | |
| JM | 58 y, M | Contusion and haematoma of left parieto-temporal region, clouded sensorium, temporospacial disorien-tation, left mydriasis. | Brain trauma.Left parieto-occipital subdural hygroma. | Left parieto temporal cortex. |
| *Case 2* | | | | |
| OP | 60 y, F | Head injury in traffic accident, fracture of both legs, state of coma, abo-lition of reflexes. Left midriasis. After recovery showed disorders of behaviour (Post-traumatic confusional syndrome). | Brain trauma. Sub-dural hygroma. | Right parietal cortex. |
| *Case 3* | | | | |
| JRCR | 80 y, M | After suffering fall, chronic alcoholic patient showed headache, di-minution of muscle strength of lower extre-mities and right arm, temporary loss of cons-ciousness, dysarthria, ani-socoria. | Left frontoparietal-occipital subdural haematoma. | Left parietal cortex. |
| *Case 4* | | | | |
| MR | 67 y, F | Brain trauma after fall. | Brain trauma. Menin-gioma. Arterial hypertension. | Frontal cortex. |

## Results

Light microphotograph of semithin sections of cortical biopsy of perifocal region of parietal cortex, stained with Toluidine blue, showed the status spongiosus that features severe brain oedema of brain parenchyma of a 58 years old patient with an associated subdural hygroma. exhibiiting necrotic and apoptotic nerve cells, astrocytes containing lipofucsin granules, dense oligodendrocytes, and degenerated myelinated axons (Fig. 1).

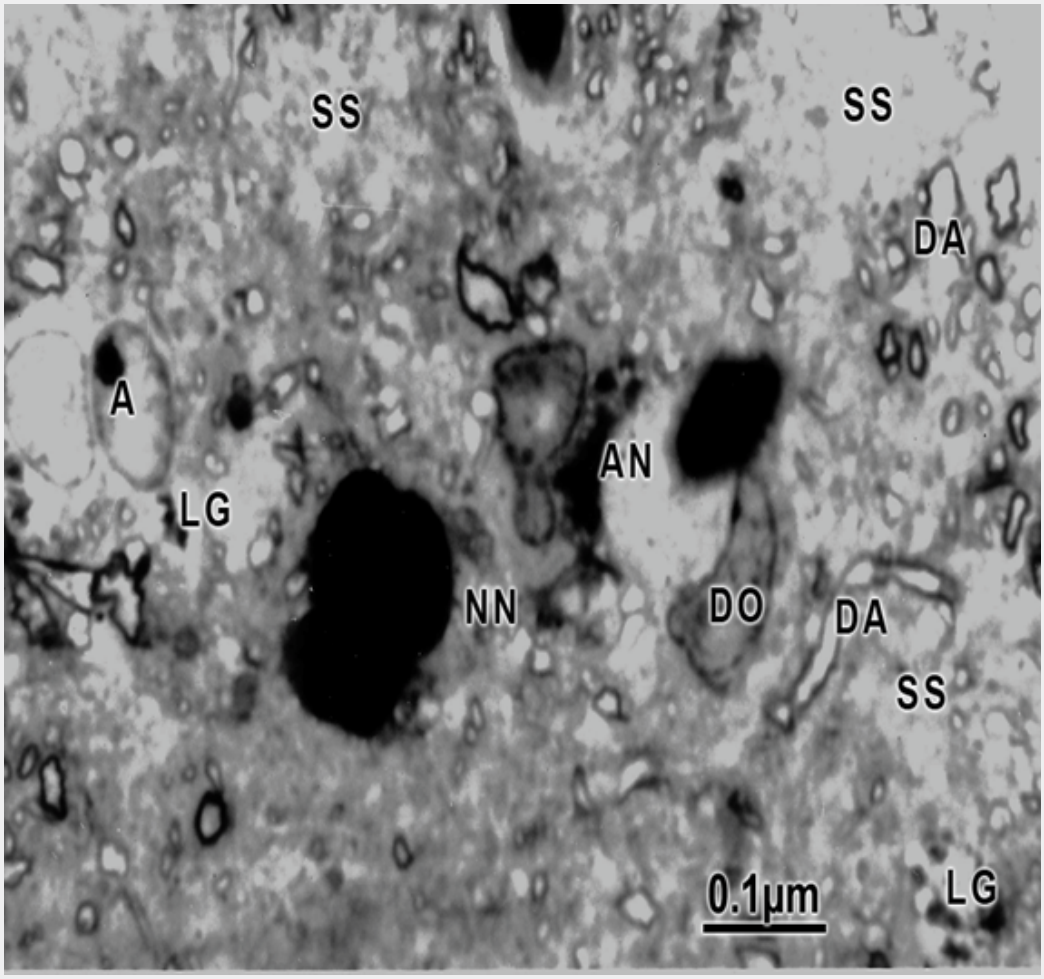


***Figure 1:*** *Brain trauma. Left parieto-occipital subdural hygroma. 57 years old patient. Severe brain edema. Light photomicrograph showing the status spongiosus (SS) of brain parenchyma, necrotic (NN) and apoptotic (AN) neurons, binucleated astrocytes (A) containing lipofucsin granules (LG), dense oligodendrocytes (OL), and degenerated myelinated axons (AX). Toluidine blue stained semithin plastic section. X 840.*

Electron micrographs of ultrathin sections stained with uranyl acetate and lead citrate of frontal cortex of a 80 years old patient with a subdural haematoma exhibited vacuolar enlargement of rough endoplasmic reticulum with detachment of associated ribosomes, a nucleus with distended nuclear pores and irregularly dilated nuclear envelope, swollen mitochondria, enlarged extracellular space, and degenerated myelinated axons (Fig. 2).

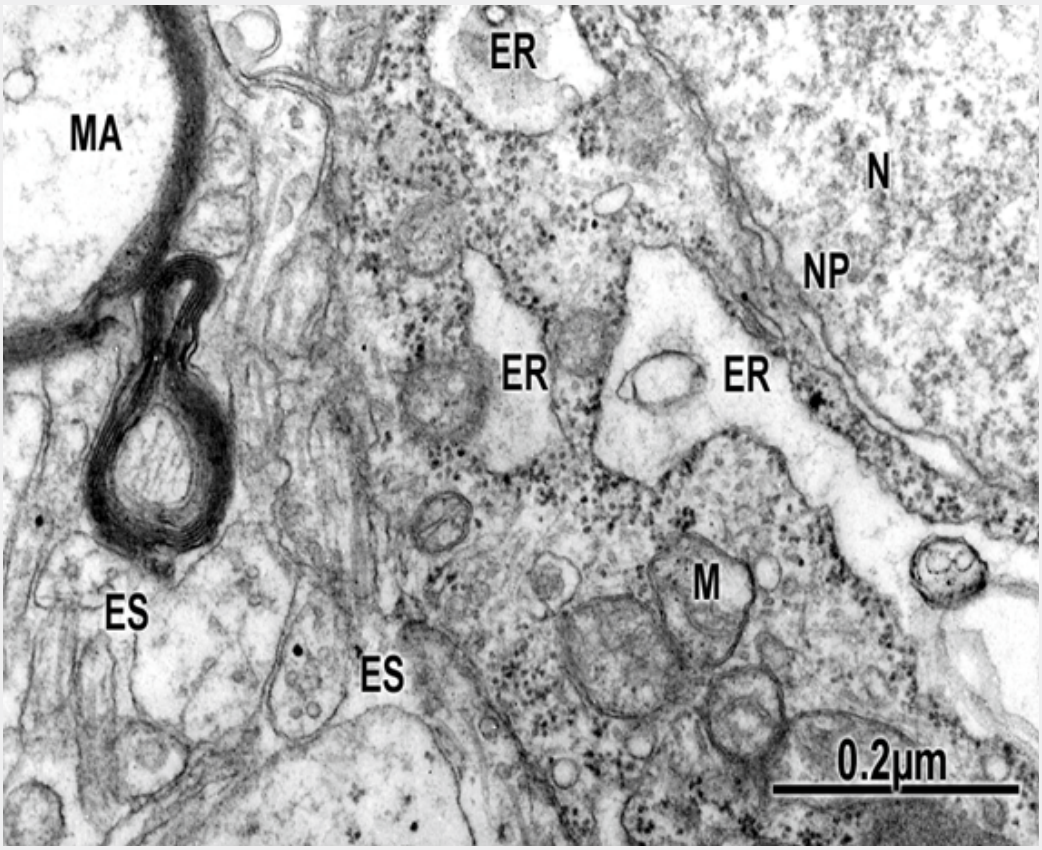


***Figure 2:*** *Brain trauma. Fronto-parieto-occipital subdural hematoma.80 years old patient. Severe edema. Electron micrograph of non ppyramidal neuron (NP) depicting vacuolar enlargement of rough endoplasmic reticulum (ER), nucleus (N) with distended nuclear pores (NP) and irregular dilation of nuclear envelope, swollen mitochondria (M). The neighboring neuropile exhibits enlarged extracellular space (ES), and degenerated myelinated axons (AX). Uranyl and lead citrate stainings. X 12000.*

A higher magnification electron micrograph of same patient depicted cristaloid arrangement of cytoplasmic membranes, apparently an especial arrangement of smooth endoplasmic, irregularly enlarged rough endoplasmic also exhibiting detachment of associated ribosomes. The perineuronal glycogen depleted-astrocytes showed clustered thin vesicular of smooth endoplasmic reticulum (Fig. 3).

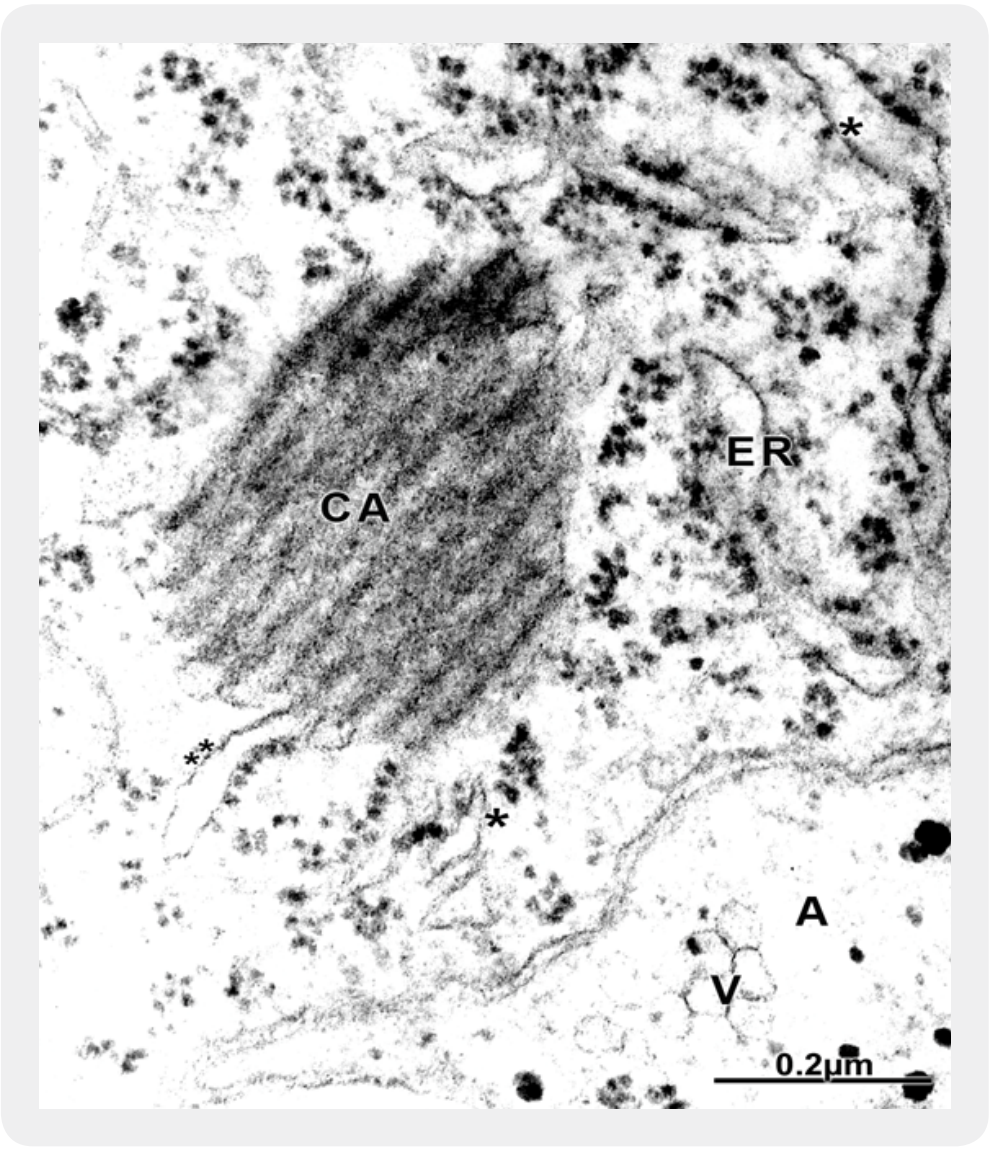


***Figure 3:*** *Brain trauma. Fronto-parieto-occipital subdural hematoma.80 years old patient. Higher magnification of cytoplasm exhibiting abnormal cristaloid arrangement of smooth cytoplasmic membranes (CA), rough endoplasmic reticulum (ER) with detachment of associated ribosomes (asterisks). The perineuronal astrocyte (A) shows vesicular (V) arrangement of smooth endoplasmic reticulum. Uranyl and lead citrate stainings. X 18.000.*

Swollen non-pyramidal neurons appeared also surrounded by glycogen rich-astrocyte cytoplasm, degenerated and isolated presynaptic endings, and degenerated axodendritic synapses. Degenerated myelinated axons contained disrupted neurofilaments, and appeared surrounded by periaxonal astrocyte (Fig, 4).

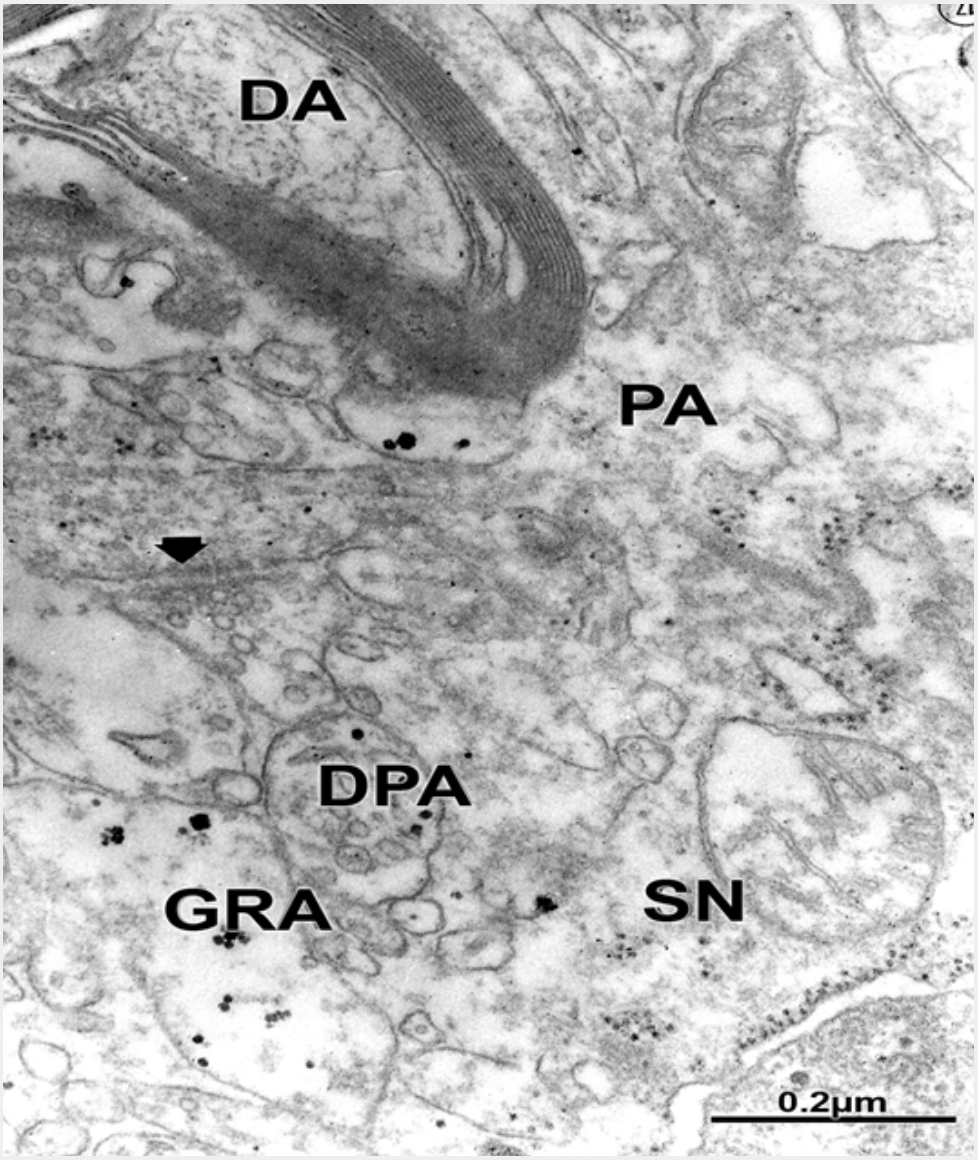


***Figure 4:*** *Brain trauma. Subdural hematoma.75 years old patient. Severe edema. Swollen non- pyramidal neuron (SN) surrounded by glycogen rich-astrocytic cytoplasm (GRA), a degenerated degenerated axodendritic synapse (thick arrow). A degenerated myelinated axon (DA) contains disrupted cytosketal structures, and appears surrounded by periaxonal astrocyte (PA) containing glycogen granules. Uranyl and lead citrate stainings. X 8000.*

In a 67 years-old patient with very severe traumatic eodema and subdural haematoma we found degenerated myelinated axons with notably vacuolization of outer myelin lamellae. These axons appeared attached to swollen and ischaemic oligodendrocytes (Fig. 5).

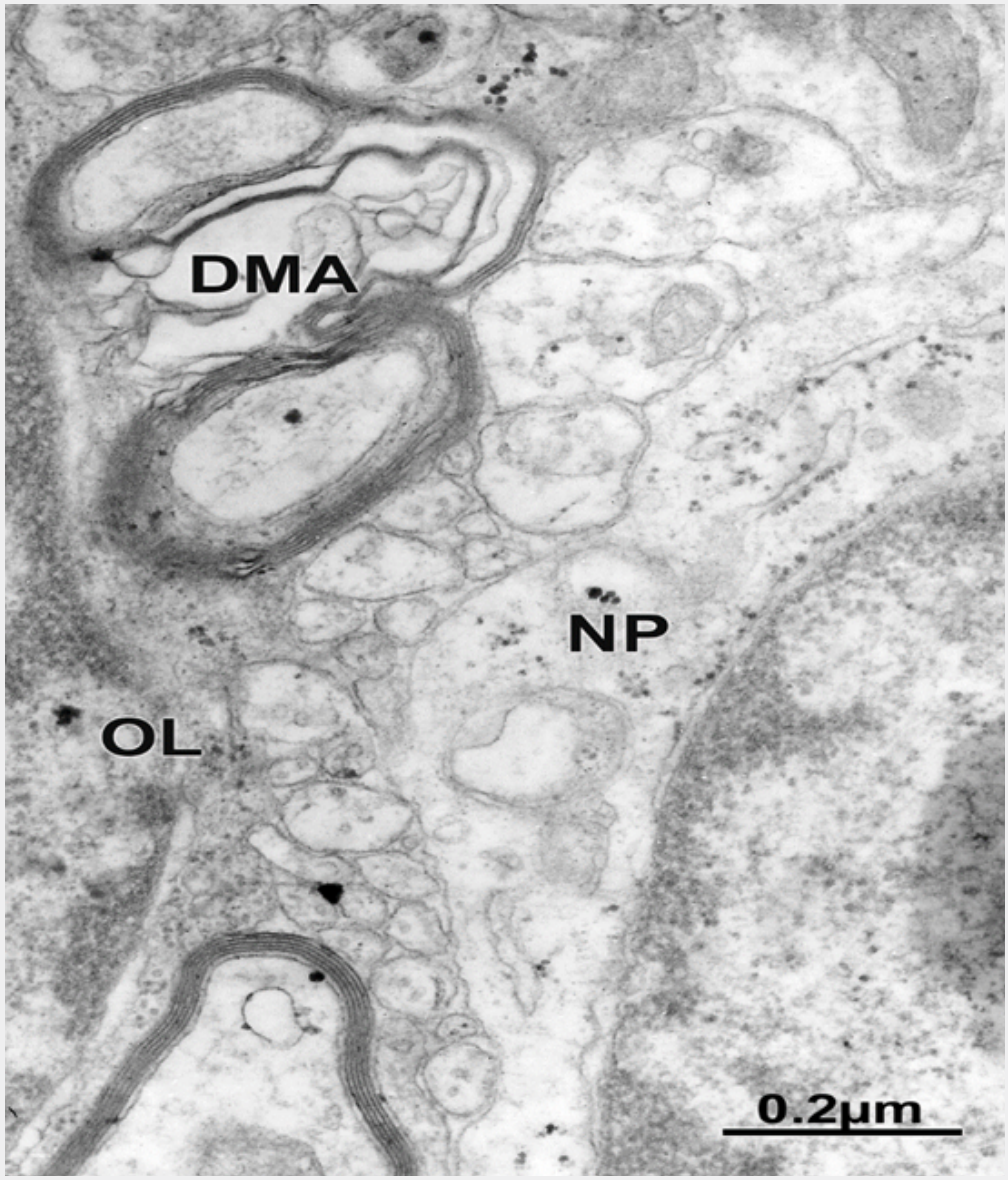


***Figure 5:*** *Brain trauma. Fronto-parieto subdural hematoma.79 years old patient. Severe edema Degenerated myelinated axon (DMA) attached to a swollen oligodendrocyte (OL) and displaying vacuolar degeneration of myelin sheath. A neighboring swollen non-pyramidal neuron (NP) also is seen. Uranyl and lead citrate stainings. Magnification = 12000.*

In a 60 years-old patient with severe brain we observed lipofucsin-rich swollen astrocytes.This finding was found in most cortical biopsies examined. (Fig, 6).

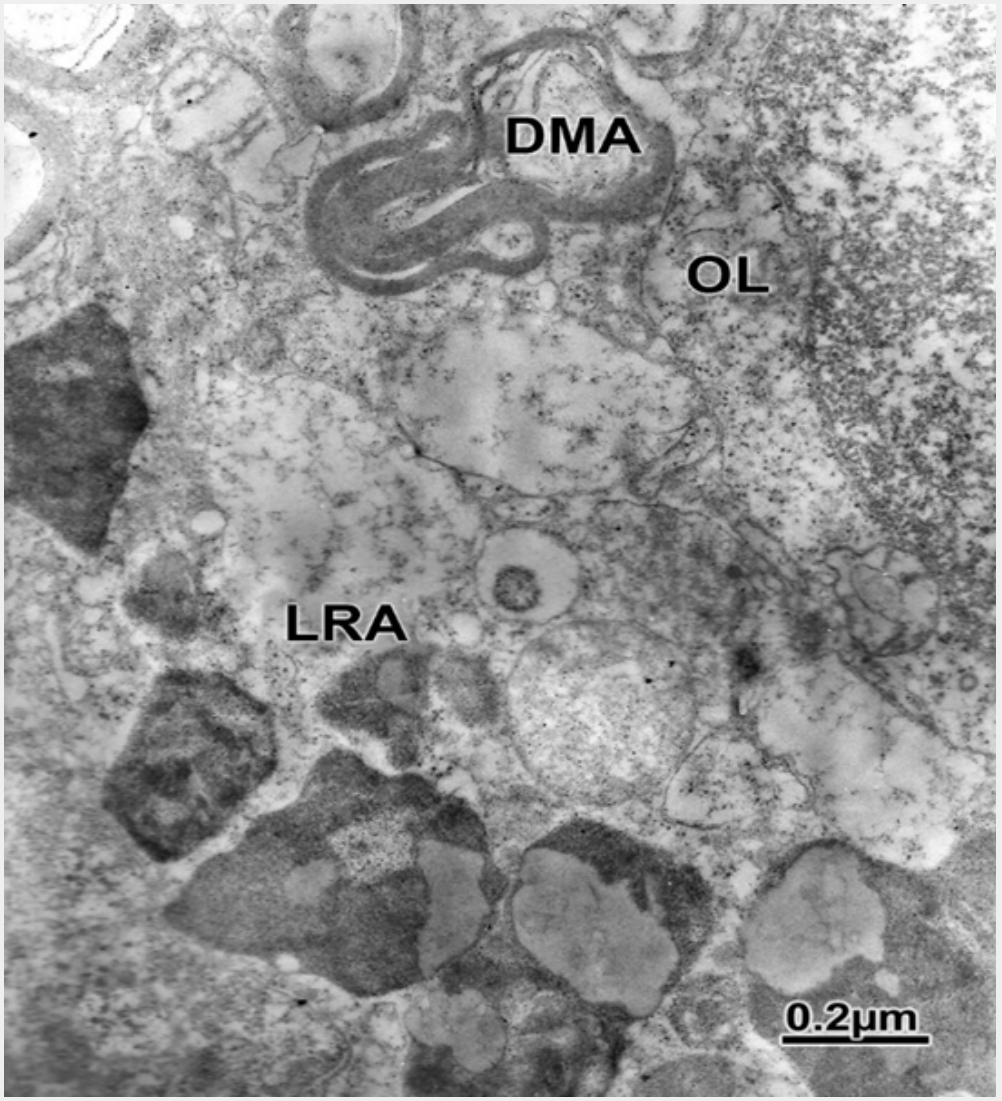


***Figure 6:*** *Brain trauma. Subdural haematoma. 60 years-old patient. Severe brain oedema. Lipofucsin-rich astrocyte (LRA) showing numerous lipofuscin granules (LG). A degenerated myelinated axon (DMA) and a severe swollen oligodendrocyte (OL) also are observed. Uranyl and lead citrate staining. X 24000.*

At the electron microscopy level of capillary wall, the blood-brain barrier breakdown was observed featured by increased surface activity of luminal endothelial cell, emission of thicks and thin microvilli, vesicular and vacuolar transendothelial transport (Fig. 7).

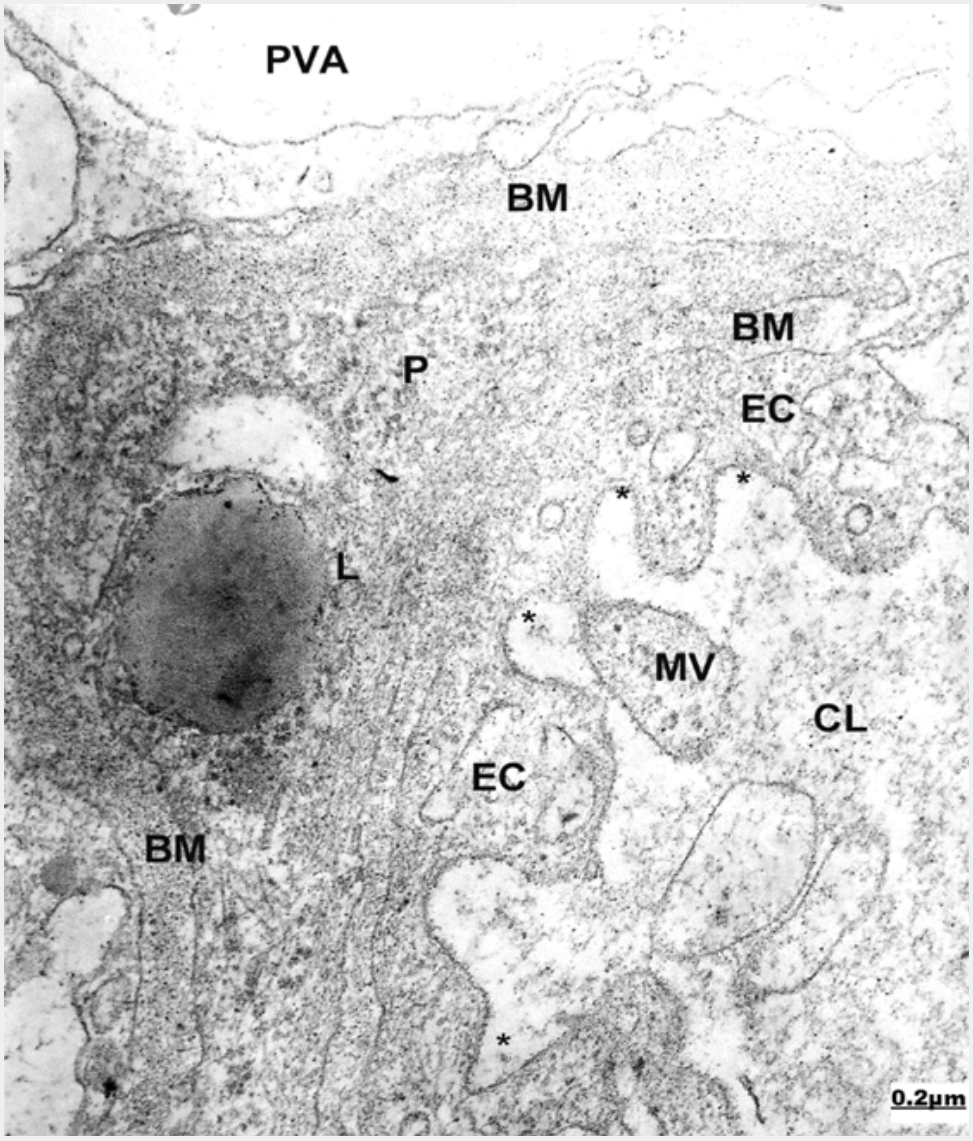


***Figure 7:*** *Brain trauma. Fronto-parieto subdural haematoma.67 years-old patient. Severe brain oedema. Capillary wall showing the capillary lumen (CL), an activated endothelial cell (EC) with thick microvilli (MV), and deep invaginations of endothelial luminal surface (asterisks). The swollen pericyte (P) embedded into the basement membrane (BM) shows a liposome (L), microtubules and microfilamnts. Uranyl and lead citrate staining. X 18000.*

In very severe traumatic injuries the basement membrane appeared thickened and vacuolated enclosing the swollen pericyte cytoplasm. A proteinaceous edema fluid appear surrounding the outer surface of basement membrane and rejecting the perivascular astrocyte cytoplasm and inducing dissociation of glio-basal unit, and blood brain barrier breakdown (Fig. 8).

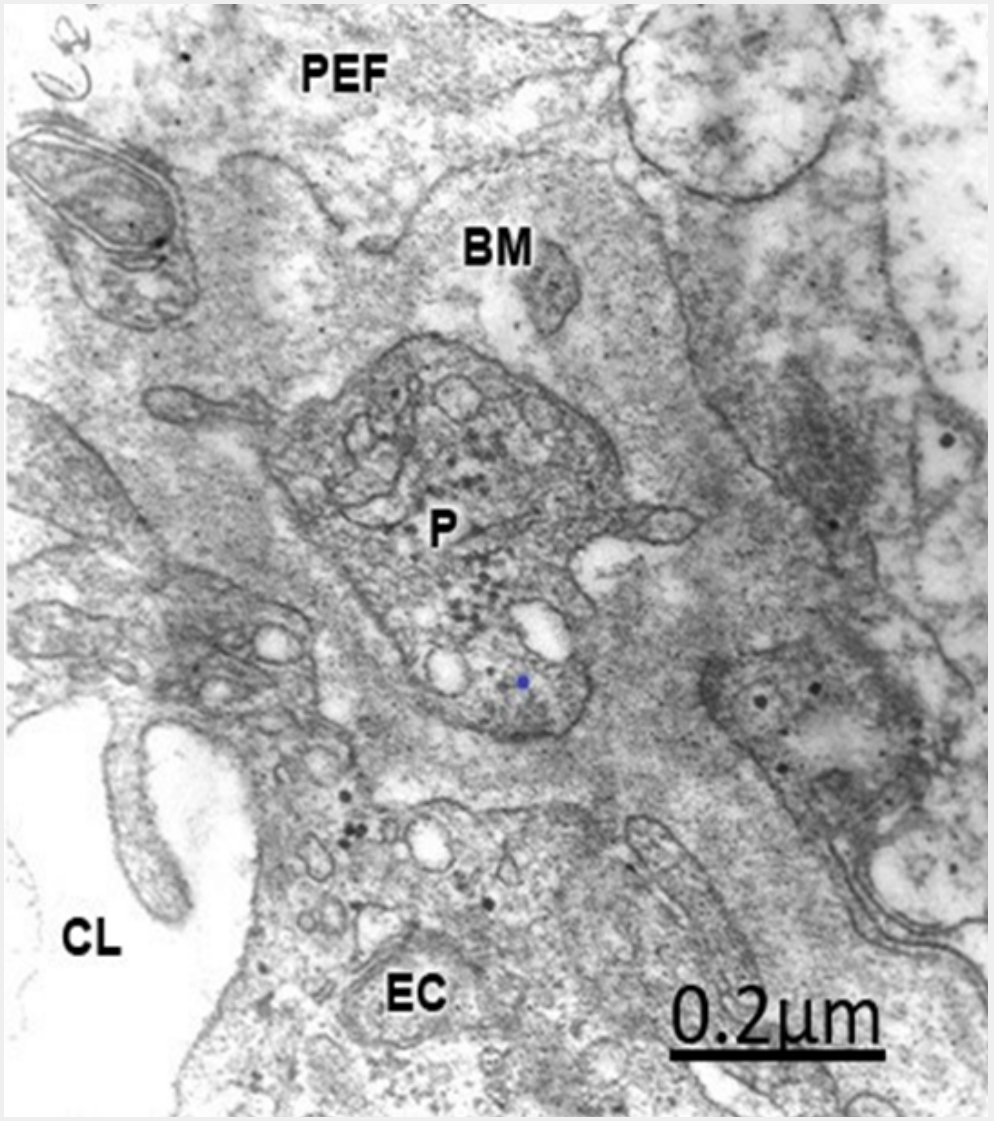


***Figure 8:*** *Brain trauma. Fronto-parieto subdural hematoma.79 years old patient. Swollen and vacuolated basement membrane (BM) enclosing the edematous pericite cytoplasm (P)- The proteinaceous edema fluid (PEF) appear occupying the space between the basement membrane and the perivasculasr astrocyte, X 36.000.*

Some capillary wall showed a multilayered basement membrane, which appear separated by thin bands of swollen pericyte cytoplasm (Fig. 9).

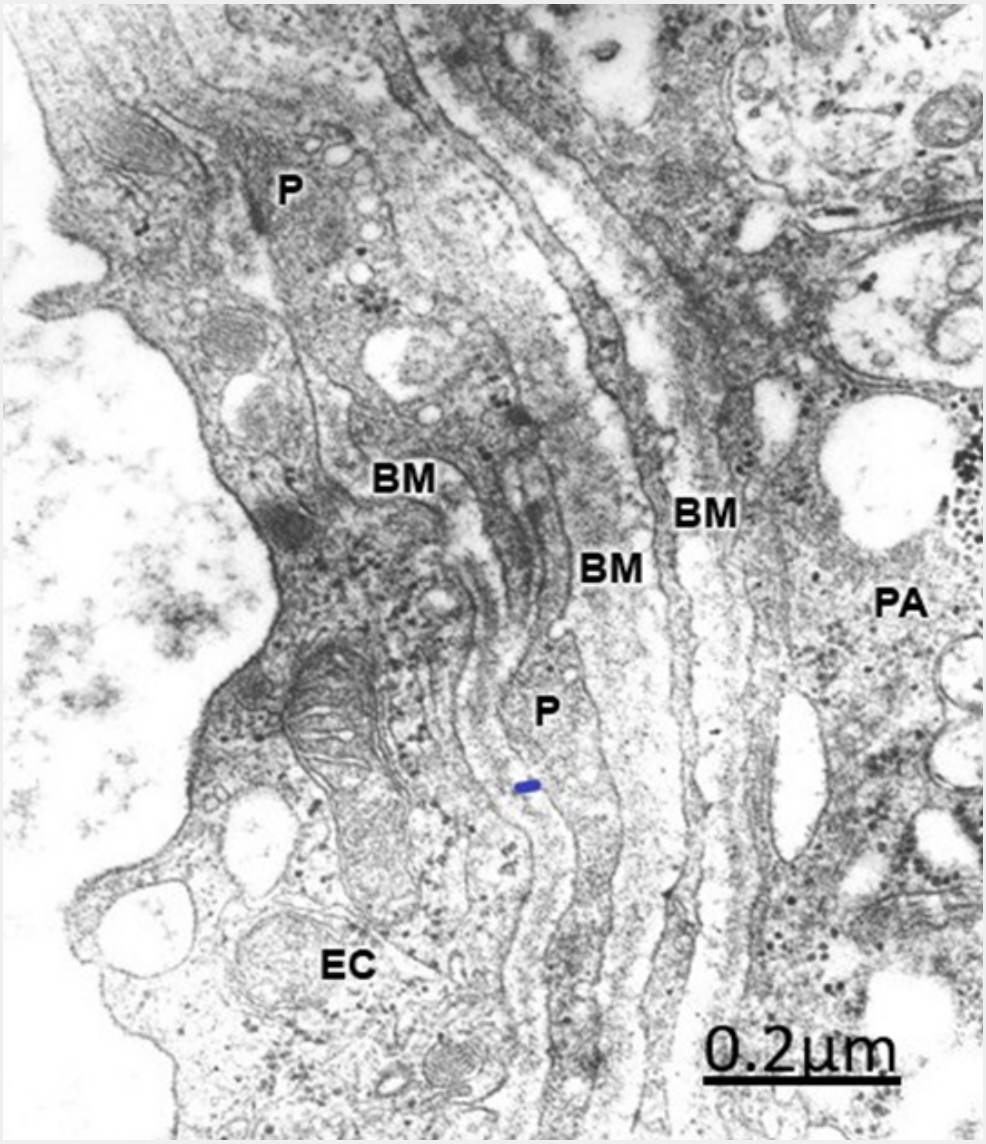


***Figure 9:*** *Brain trauma. Subdural haematoma. 60 years-old patient. Severe brain oedema. Capillary wall showing a multilayered basement membrane (BM) separated by swollen pericyte cytoplasm (P) showing proliferation of collagen fibers (short arrows). A dense perivascular astrocyte (PA) appears attached to the basement membrane (BM). X 36000.*

The multilayered basement exhibited proliferation of collagen fibers with attached perivascular dense microglial cells also were found (Fig. 10).

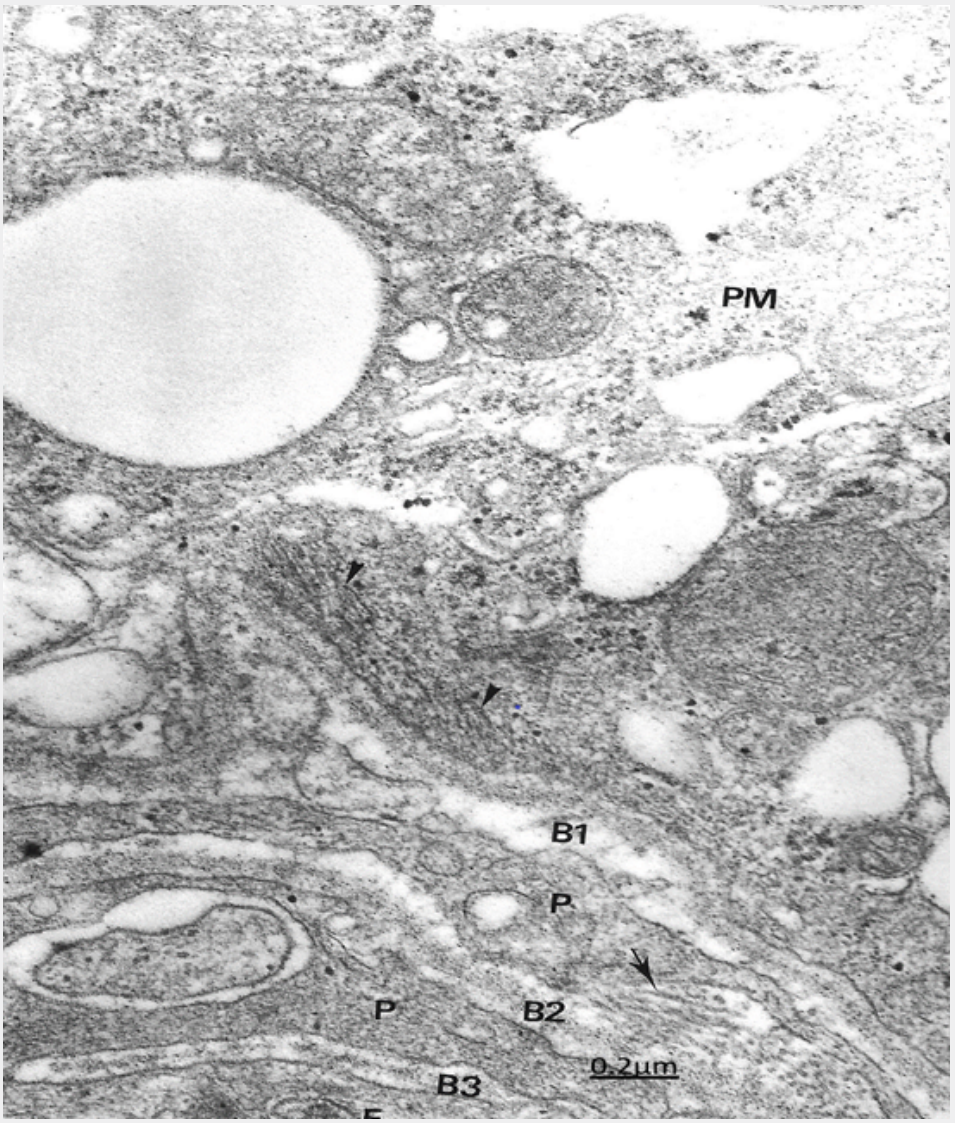


***Figure 10:*** *Brain trauma. Subdural haematoma. 60 years-old patient. Severe brain edema. Capillary wall showing a multilayered (B1, B2, and B3) basement membrane (BM) separated by swollen pericyte cytoplasm (P). A proliferation of collagen fibers (short arrow) is observed attached to the outer surface of the basement membrane (B2). A dense and vacuolated perivascular microglial cell cytoplasm (PM) appears attached to the basement membrane (BM1) by means of anchored microfibrils (arrowheads). X 36000.*

## Discussion

In the present study we have systematically examined the perifocal regions of traumatically injured swollen cerebral cortex with associated subdural hygroma or hematoma of patients with ages ranging from 57 to 80 years old. Acute subdural hematoma was one of the conditions most strongly associated with severe brain

injury. White matter (WM) undergoes a series of changes during the process of aging. WM malfunction can induce serious neurobehavioral and cognitive impairments. Thus, age-related changes in WM may contribute to the functional decline observed in the elderly. In addition, aged WM becomes more susceptible to neurological disorders, such as stroke, traumatic brain injury (TBI), and neurodegeneration [15].

We observed stress endoplasmic reticulum (ER) of nerve cells induced by the shear forces exerted by the physical trauma intensity [16]. ER stress and impaired autophagy flux in neuronal degeneration. Brain injury has been recently reported by Yang *et al.* [17] (2015) and Yin *et al.* [18] (2017) in ethanol neurotoxicity. According to these Authors, ER stress has been implied in various CNS injuries, including brain ischemia, traumatic brain njury, and aging-associated neurodegeneration, such as Alzheimer's disease (AD), Huntington's disease (HD), Amyotrophic lateral sclerosis (ALS), and Parkinson's disease (PD).

In addition, we have herein observed detachment of associated ribosomes and specialized arrangement of smooth endoplasmic reticulum, apparently induced by the shear forces and intensity of traumatic agent. Such ribosome detachment could partially explain the transitory memory loss of patients with severe head injuries [19].

We have found here in reported edematous mitochondria. There is an interplay between mitochondrial DNA, pathophysiology of TBI, and aging. Structural and functional damage of mitochondria is currently found in traumatic brain injuries [20-24].

Mitochondria contain their own DNA (mtDNA) with genomic variants that have different physiological and pathological characteristics, including susceptibility to neurodegeneration. Given the central role of mitochondria in the pathophysiology of neurological injury, Bulstrode *et al.* [25] (2014). hypothesized that its genomic variants may account for the variability in outcome following TBI. The data also suggest that the APOE pathways interact with genetically regulated mitochondrial functions in the response to acute injury, as previously reported in Alzheimer disease.

We have also distinguished glycogen-rich astrocytes as satellite of ischemic neurons, oligodendroglial, microglial cells and synapses. This astrocyte type has been previously reported by us [26], and we have postulated its metabolic contribution for mantaining and survival of neurons and glial cells in conditions of energy and metabolic substrates limitations.

Some biochemical events should be considered in relation to traumatic brain edema, such as oxygen radical generation and lipid peroxidative reactions [27-30] , glutamate release during ischemia and activation of NMDA re¬captor [31], increase in intracellular $Ca^{2+}$ concentration [32] free radical generation and increased concentration of polyamines in the brain [33], disturbance in ion homeostasis involving cellular release of $K^{+}$ and massive $Ca^{2+}$ entry into the intracellular compartment as occur in brain edema [34].

In relationship with the degeneration of myelinated axonal, we have earlier reported varicose fiber swelling, myelin sheath distortion, formation of myelin ovoids, and increased amount of oligodendroglial ad-axonal layer. We observed myelin degeneration, redundant myelin sheaths, and dense oligodendrocytes, Some pathogenetic mechanisms on central axonal degeneration has been earlier proposed by us [35].

We have found increased transendothelial vacuolar transport [36], basement membrane thickening and swollen pericites. The presence of multilayered basement membrane and proliferation of collagen fiber could be explained by repeated hypoxic events [37,38].

Light and electron microscopy studies showed apoptotic necrotic cell death of nerve and glial cells, which suggested intrinsic cortical disruption and degeneration Sustained ER stress may result in cell death. Excitotoxicity also is involved in nerve cell death [38,39].

We have recently described the blood brain barrier disruptions on brain aging induced by severe and complicated brain injuries [40].

## Conclusions

Severe human brain traumatic injuries in elderly patients induced blood brain barrier breakdown, primary vasogenic edema and secondary cytotoxic edema. Light and electron microscopic study showed status spongiosus of brain parenchyma, apoptotic necrotic and autophagic nerve cell death types, reactive hypertrophic astrocytes containing lipofucsin granules, dense oligodendrocytes, and degenerated myelinated axons. Satellite perineuronal, interfascicular and perivascular glycogen rich and glycogen- depleted astrocytes. Astrocytes showed clustered and thin vesicular profiles of smooth endoplasmic reticulum. The degenerated myelinated axons appeared attached to swollen and ischemic oligodendrocytes. Close examination of the neuropile showed synaptic plasticity and synaptic degeneration. A close relationship between severe traumatic injuries, brain aging and neurodegenerative diseases is emphasized.

## Acknowledgements

This paper has been carried out by the logistic support of Biological Research Institute and Castejón Foundation.

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