UNIVERSITY OF MEDICINE AND PHARMACY "CAROL DAVILA", BUCHAREST

DOCTORAL SCHOOL

FIELD OF FORENSIC MEDICINE

SUMMARY OF THE DOCTORAL THESIS

SCIENTIFIC COORDINATOR

PROF. UNIV. DR. CURCĂ GEORGE CRISTIAN

PhD candidate:

CHIRICĂ VIOLETA-IONELA

2022

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NECROPSY EVALUATION OF BRAIN INJURY SEVERITY AND POST-TRAUMATIC HYPOXIA

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INTRODUCTION

The incidence of traumatic brain injury (TBI) is steadily increasing year by year, representing one of the most common causes of death as well as a critical public health and socio-economic problem [1, 2].

Traumatic brain injury is defined as "an alteration in brain function or other evidence of brain pathology caused by an external force" [3].

Clinically, the most widely used method of assessing the severity of traumatic brain injury is the Glasgow Coma Scale (GCS) which classifies TBI into mild, moderate and severe; this classification is also useful in assessing prognosis for survival [4].

Traumatic brain injury is characterized by great heterogeneity in terms of etiopathogenesis, biomechanics, pathophysiological mechanism of evolution, severity and treatment. Primary brain lesions are progressive, being aggravated by both intrinsic and systemic pathophysiological mechanisms [3-6].

Deaths caused by traumatic brain injury occupy an important part of forensic practice, in these cases a forensic autopsy is mandatory under Romanian law, Article 185 of the Code of Criminal Procedure. Forensic expert has to determine the cause of death and the manner of death, the mechanism of the cranio-cerebral injuries and their contribution to the chain of events that lead to the death.

In order to be able to provide objective scientific evidence of the causal or contributory nature of a cranio-cerebral injury in causing death, the forensic autopsy must target both primary brain injuries and secondary progressive injuries. Given the high mortality rate among traumatic brain injuries, studies on the circumstances of brain injury, the timing of traumatic injury, the biomechanical mechanism of traumatic injury and the pathophysiological mechanism of traumatic injury are still relevant.

At the same time, in order to correctly asses the severity of the traumatic brain injury and its contribution to the tanatogenesis chain, it is necessary for the forensic necropsy to demonstrate the morpho-pathological changes induced by cerebral hypoxia, an aspect which is also of considerable interest in forensic practice.

The present study aims to assess the severity of traumatic cranio-cerebral injuries encountered in forensic practice through a descriptive analysis of demographic and

epidemiological characteristics as well as morphological features both macroscopically and microscopically through histopathological and immunohistochemically investigations of brain tissue.

Objectives of the study

1. To establish the characteristics of cranio-cerebral injuries, the mechanism of their occurrence and the circumstances of their occurrence in relation to forensic autopsy findings in fatal TBI cases.
2. Descriptive analysis of the severity of cranio-cerebral injuries in relation to the clinical data recorded in the clinical observation sheets of fatal TBI cases, as well as the necropsy findings.
3. Comparison of macroscopic necropsy findings of traumatic brain injury with imaging findings diagnosed by CT examination performed ante-mortem.
4. Assessment of the severity of the brain lesions by histopathological microscopic analysis using the usual hematoxylin-eosin staining performed on tissue samples taken from the lesion, the contralateral foci and the hippocampus ipsilateral to the traumatic lesion.
5. To study the expression of the immunocytochemical and functional response of cortical brain injury using antibodies specific to structural proteins in neurons, (Neurofilament and β-APP) by comparing deaths following traumatic brain injury with deaths from other non-cerebral causes.
6. To study the expression of astrocytes following traumatic injuries by their immunohistochemically analysis using GFAP staining in relation to the TBI cases studied and the control cases.

The present work is structured in two parts. The first part is the general part and includes a description of the theoretical aspects of traumatic brain injury as well as the current status of the topic under investigation, obtained after a laborious bibliographic documentation.

The second part is the personal contribution part which includes the research methodology, results, discussions and conclusions of the PhD study.

GENERAL PART

CHAPTER 1. EPIDEMIOLOGY, DEFINITION AND CLASSIFICATION OF TRAUMATIC BRAIN INJURIES: This chapter presents data from the most recent epidemiological studies on traumatic brain injury, describing the incidence and mortality among people suffering annual traumatic brain injury, both in Europe and globally [4,7]. At the same time, the most commonly used definitions of traumatic brain injury are presented, focusing on the most recent definition of traumatic brain injury formulated by Menon (2010) and the criteria used by him in defining TBI, such as alteration of brain function and clinical signs that characterize it, the existence of neuroimaging confirmed brain damage and the mechanism of production of brain injury. This chapter also presents the clinical, imaging and morphological classifications of traumatic brain injury and the biomechanical elements involved in determining traumatic effects.

CHAPTER 2. BRAIN TRAUMATOLOGY: this chapter is divided into 9 sub-chapters, in each sub-chapter the theoretical aspects of primary and secondary brain injuries are presented, for each type of injury the particular epidemiological aspects are described, the pathophysiological mechanisms of occurrence and evolution, the clinical elements in establishing the diagnosis, severity and prognosis, the imaging diagnostic elements as well as the necropsy, macroscopic and microscopic aspects, in relation to the severity of brain injuries and their temporal evolution. Knowledge of these aspects is of particular importance in forensic practice, the forensic pathologist being the one who, by corroborating the ante-mortem clinical aspects with the post-mortem aspects objectified necropsy, must establish the mechanism of production of traumatic brain injuries and their contribution in the tanatogenesis.

CHAPTER 3. NEUROPATHOLOGY OF TRAUMATIC BRAIN INJURIES is divided into two subchapters. The first part focuses on the description of post-traumatic brain hypoxia in terms of pathophysiological mechanisms and cellular changes following hypoxia. Due to high energy consumption and metabolic activity, the brain has an increased sensitivity to hypoxia and hypoperfusion, hypoxic-ischemic lesions being the most common causes of cell necrosis, the most severe neurological dysfunctions being the consequence of hypoxia-hypoperfusion association. The presence of cerebral hypoxia has been reported in

approximately 44% of traumatic brain injury patients with brainstem injury and impaired cerebral perfusion, as well as in extracranial trauma patients [8]. The most vulnerable areas to hypoxia are the hippocampus, especially the CA1 region (Sommer sector), followed by the CA4 region and the least vulnerable, the CA2 region, the basal nuclei, globus pallidus and putamen, the pyramidal neurons of the cerebral cortex and the Purkinje cell layer of the cerebellum [9]. Histological changes caused by hypoxia can best be observed in cortical layers three, five and six. Due to high metabolic needs, neurons are more sensitive to hypoxia than macroglia.

The second sub-chapter, entitled DIAGNOSTIC AND PROGNOSTIC BIOMARKERS OF BRAIN TRAUMATIC INJURIES, presents a selection of markers with potential for clinical use based on the anatomical location of lesions and mechanisms of production following brain trauma, such as axonal lesions, acute neuronal lesions, acute astroglial lesions, neuroinflammation and amyloid-related processes. In general biomarkers can be classified as diagnostic, prognostic and predictive markers but their main characteristics are sensitivity and specificity. As far as brain biomarkers are concerned, some of them have been shown to be useful as both diagnostic and prognostic markers [10]. At the same time, recent post-mortem studies suggest that these biomarkers used in clinical practice have a high potential in the necropsy assessment of brain injury severity.

II. PERSONAL CONTRIBUTION

In cases of suspected TBI where necropsy findings do not reveal traumatic injuries or in cases of minor head trauma, a thorough histopathological evaluation is necessary to establish a traumatic cause of death.

The aim of this study is to describe the characteristics of fatal traumatic brain injuries both necropsically and histopathologically, to analyze the severity of traumatic brain injuries in relation to clinical medical data and necropsy findings as well as to analyze the severity of traumatic injuries on microscopic preparations using the usual stains and relevant immunohistochemical stains.

The results of the present research are based on three studies carried out at INML "Mina Minovici" Bucharest, 2 studies on different batches and a case study, for which the Research Ethics Commission of INML, Mina Minovici, obtained the approval.

1. The first study, **NECROPTIC ASPECTS OF TBI CASES AUTOPSIATED AT THE NATIONAL INSTITUTE OF LEGAL MEDICINE, MINA MINOVICI, BUCHAREST, DURING THE PERIOD JANUARY 2017-DECEMBER 2018**, consists of a retrospective, observational study, based on the evaluation and analysis of 3835 cases of medico-legal autopsy, from which 479 violent deaths were selected whose cause of death was represented by cranio-cerebral trauma.

Cases of polytrauma that also involved traumatic brain injury, but which were not involved in the tanatogenesis or had a minor contribution and were therefore not recorded in the medical certificate of death, were not included in the study.

Cases that were initially suspected to be traumatic brain injury but for which an autopsy identified a pathological brain cause were excluded from the study.

The aim of this study was to describe the characteristics of fatal traumatic craniocerebral injuries revealed during medico-legal autopsies performed in INML Bucharest between January 2017 and December 2018.

For data collection, necropsy reports were analyzed, and for cases that required hospitalization prior to death, complete observation sheets and radio-imaging investigations performed during hospitalization were also analyzed.

The following data were collected from the completed autopsy reports in the archives of the Clinical Forensic Medicine Laboratory and Prosectura II:

- epidemiological and demographic data (age, sex),
- data on the circumstances and mechanism of traumatic injuries,
- clinical data (GCS score on admission, duration of hospitalization, clinical aspects identified during radio-imaging investigations and clinical medical examination),
- macroscopic traumatic aspects identified at necropsy,
- cranio-cerebral histopathological aspects resulting from the microscopic analysis requested during the autopsy,

Data were collected using Microsoft Excel -Office 2019 and analyzed using Microsoft Excel-Office 2019 and IBM SPSS Statistics 24.0

Results

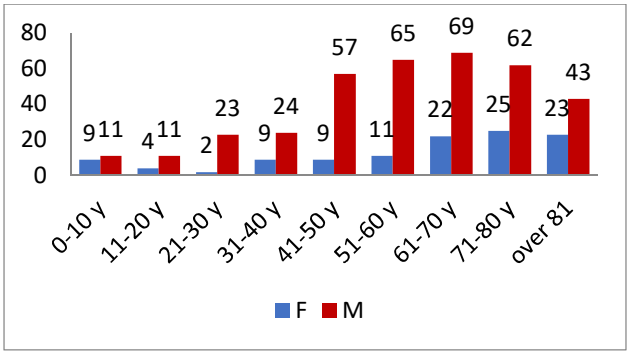
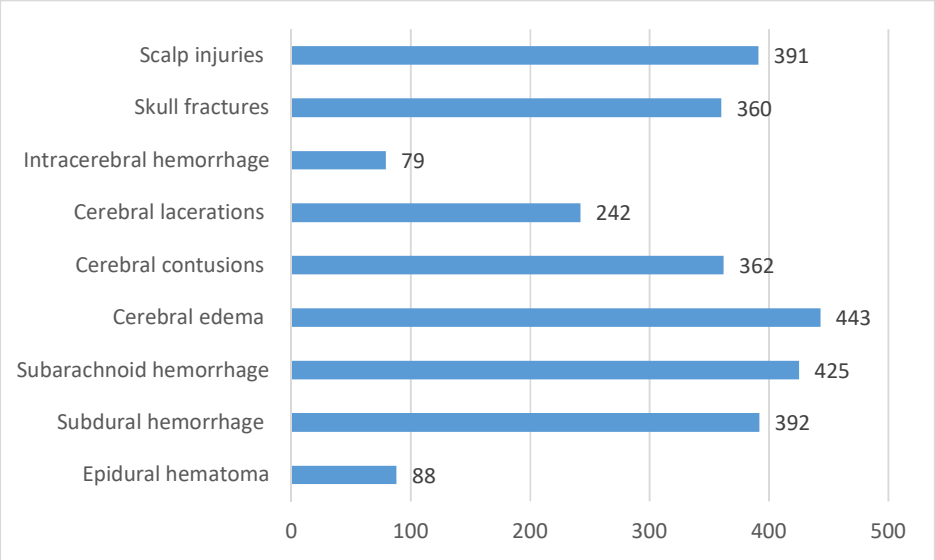


Fig 6.4.Graphical representation of the distribution of cases studied by age group and gender

In the studied group, the male population prevailed, with a male: female ratio of 3:1, aspects also found in other epidemiological studies carried out [4]. In this study, the average age of people who suffered a TBI was 58 years, with extremes ranging from 2 to 97 years, with a maximum reached between ages 61 and 70. This supports the theory of changing age pattern described by Roozenbeek [11] from younger ages to the elderly population, with TBI now affecting this group more frequently as a result of falls. Currently the presence of mild TBI is a significant risk factor for death, especially in the elderly [12].

The main circumstances of traumatic brain injury are road traffic accidents and falls from the same level. In the studied group, 120 cases were diagnosed at the time of admission to the medical unit as "TBI in unspecified conditions". Subsequent investigation data correlated with necropsy findings showed that in these cases the traumatic injuries were produced as a result of falls from the same level, which means, that in the studied group the fall is the main circumstance of production of traumatic injuries. The rarest situations of traumatic brain injury were gunshots and TBI due to explosions (Fig 6.8).

We observed that road traffic injuries were more common in young people under 50 years of age, while falls were the main circumstance of TBI in people over 60 years of age [13]. This observation is similar to Peeters' observation that there is a correlation between age groups and circumstances of TBI occurrence [14].



Necropsy examination of the TBI cases studied showed that the most common traumatic craniocerebral injuries were subarachnoid hemorrhage (88%), subdural hemorrhage (82%), followed in close percentages by cerebral contusions (76%) and skull fractures (75%). The least common were intraparenchymal hemorrhage and extradural hematoma.

The most common type of skull fracture encountered was the linear irradiated vault fracture at the base of the skull, which was present in 202 cases out of the total 360 fractures diagnosed and the least common was the isolated skull base fracture, which was diagnosed in only 11 cases. Microscopic examination most commonly diagnosed the presence of cerebral oedema in varying degrees and subarachnoid hemorrhage. At the same time, reactive gliosis, present in 65/231 cases, and neuronal ischemia, present in 52 cases out of 231 histopathological examined, were identified on microscopic slides.

Comparative analysis of the traumatic lesions showed differences in their diagnosis by CT examination performed ante-mortem and macroscopic examination performed at necropsy. Thus, in some cases where traumatic brain injuries were diagnosed by CT examination, they were not identified at the time of forensic autopsy. At the same time, the necropsy examination established the presence of previously undiagnosed injuries or disproved the presence of some, such as skull fractures [13]. This difference in results may be due to the location of the fractures, CT diagnosis of anterior fossa fractures being more difficult. In the present study we found that in 44 cases CT examination performed at the time of hospital admission did not diagnose the presence of cerebral contusions although they were macroscopically identified on necropsy.

STUDY 2: CONTRIBUTIONS ON MICROSCOPIC DIAGNOSIS OF BRAIN INJURY SEVERITY AND CEREBRAL HYPOXIA

The complexity of traumatic brain injury sometimes makes it difficult to discriminate the contribution of a particular type of brain lesion in the tanatogenesis chain, especially in cases of minor traumatic brain injury or suspected TBI where necropsy does not reveal macroscopic brain lesions.

Brain injury following traumatic brain injury initiates multiple complex processes between neurons and glial cells leading to the development of secondary brain lesions with a role in restoring brain plasticity and remodeling. Disturbances in the communication pathways between neurons, astrocytes and microglia represent a pathophysiological mechanism of neurodegeneration that also occurs in response to hypoxic-ischemic processes following trauma.

Immunohistochemical analysis has proven its usefulness over time in determining the cause of death and is thus an important method of investigation in forensic practice. Immunohistochemical studies reveal not only cellular changes in the injured brain tissue but also global secondary changes.

The expression of glial fibrillary acidic protein (GFAP) in injured tissue is considered to be a useful marker in the identification of reactive astrocytosis. Mature and differentiated astrocytes contain dense networks of GFAP intermediate filaments, which immediately after injury release GFAP into the extracellular space and surrounding interstitial fluid, while becoming reactive with rapid GFAP production to maintain cellular integrity. The role of GFAP is to maintain astrocyte stability, essential in reactive processes such as astrogliosis and glial scar formation [15].

Increased GFAP expression post-trauma may result from damage to glial filaments as a consequence of brain edema or GFAP synthesis by fibrotic astrocytes located in layers II-VI of the cerebral cortex [16]. Typically, most protoplasmic astrocytes in the grey matter do not express sufficient GFAP to be detected by routine microscopic investigations, so these astrocytes, with the exception of the CA4 region of the hippocampus are negative [17].

Intermediate filaments or neurofilaments, components of the cytoskeleton, are synthesized at the level of the neuronal body and are found both in the neuronal soma and in the dentrite and axon. Mechanical deformations of brain tissue caused by head trauma affect the structure of the cytoskeleton and thus of axoplasmic transport in brain tissue, these changes are more frequently

observed in areas of contusion and are usually accompanied by diffuse changes in neurons located in the cerebral cortex and hippocampus.

Intermediate filaments or neurofilaments, components of the cytoskeleton, are synthesised in the neuronal body and are found both in the neuronal soma and in the dentria and axons. Mechanical deformations of brain tissue caused by head trauma affect the structure of the cytoskeleton and thus the axoplasmic transport by ballooning of axons and focal accumulation of neurofilaments [18].

β-APP is a surface protein involved in intracellular neuronal processes, cell adhesion and response to trauma. β-APP can be revealed by immunohistochemical staining in axons damaged as a result of traumatic brain injury by accumulation at this level consequent to inhibition of axoplasmatic transport [19].

Objectives

- Target the reactive response of astrocytes and neurons to brain trauma, labelled with primary antibodies β-APP, GFAP and Neurofilament.

-To analyze histopathological and immunohistochemical microscopic changes in the hippocampus and to reveal a possible correlation with the severity of traumatic brain injury.

- Establishing possible correlations between immunohistochemically described cellular changes and survival time.

This study is a prospective study, conducted at the National Institute of Forensic Medicine, Mina Minovici, Bucharest, in which a series of 27 forensic cases autopsied during 2021 were analyzed. The 27 cases were subject to judicial investigations under the Romanian Code of Criminal Procedure, carried out at the request of the criminal investigation bodies, so that tissue sampling was necessary to establish the cause of death and consent from the relatives was not required. The data collected and used in the study for each case do not allow the identification of the person.

The 27 cases were divided into two groups Group I study - includes 21 cases of traumatic brain injury produced under different conditions, with macroscopic features of varying severity and varying survival duration, from immediate death to 20-day survival. These cases were divided into 3 subgroups according to survival duration in:

- 6 cases of traumatic brain injury with acute death (less than 2 hours survival from the time of the head injury)

- 5 cases of traumatic brain injury with sub-acute death (survival time between 2 and 72 hours from the time of injury)

- 10 cases of traumatic brain injury with late death (survival time over 72 hours)

Exclusion criteria group I: no cases of traumatic brain injury with neurosurgical interventions, severe autolysis or onset of putrefaction were included in the study. Study group II - control group - 6 cases as follows: 2 cases of violent death by mechanical hanging and 4 cases of non-violent death - sudden cardiac death with immediate death.

Exclusion criteria group II: cases for which cardiopulmonary resuscitation measures were applied; cases of sudden death with a history of traumatic brain injury in the recent history.

For each case included in group I, 3 fragments of brain tissue were taken: one fragment from the focus at the impact site; one fragment of tissue located at a distance from the impact site, in the contralateral focus; one fragment from the hippocampus ipsilateral to the traumatic injury.

For the cases included in group II of the study (control group), 2 tissue fragments were collected, from the right frontal lobe and the right hippocampus.

Immunohistochemical analysis was performed using anti-GFAP, anti-Neurofilament, anti-β-APP antibodies.

The study consists of two parts, a descriptive part of the H-E, anti-GFAP, anti-NF and anti-β-APP staining reported to the subgroups of group I and the control group and a statistical analysis of the data obtained from the histopathological analysis, focusing on the intensity and distribution of the immunohistochemical expression in relation to the studied groups and the macroscopic lesions.

	Acute TBI	Subacute TBI	Late TBI	Control group
Gender M F	 5/6 (83.3%) 1/6 (16.7%)	 3/5 (60%) 2/5 (40%)	 8/10 (80%) 2/10 (20%)	 4/6 (66.7%) 2/6 (33.3%)
Age Median [Interval]	60.5 [27.0, 73.0]	68.0 [16.0, 75.0]	68.5 [23.0, 80.0]	49.5 [36.0, 61.0]
Survival time (hours) Medie[interval]	0.87 [0.0, 1.83]	18.0 [3.0, 72.0]	348.0 [96.0, 480.0]	0.0 [0.0, 0.0]
PMI Medie[interval]	22.0 [16.0, 72.0]	72.0 [8.0, 96.0]	48.0 [48.0, 144.0]	22.0 [16.0, 48.0]

Table 7.1. Characteristics of the study population

Microscopic examination of the TCC cases compared to the control group reveals

hypoxia-induced changes in neurons, ranging from early changes such as cytoplasmic microvacuolization of neurons and evidence of cytoplasmic pallor to evidence of intensely eosinophilic (red neurons), in all TBI cases both at the lesion level and at the level of the fragment harvested from the contralateral lobe, compared to the control group, where the presence of neurons with hypoxia-induced changes was evidenced in 66.7% of cases. Differences were also found in the hippocampal fragment between all groups belonging to the CBT group compared to the control group, with the most hypoxic changes being observed in the CBT group with death between 2 and 72 hours (80% of cases) while in the control group hypoxic neuronal changes in the hippocampus were observed in only 33.3% of cases.

Reactive gliosis was observed microscopically in the usual H-E staining most at 18 hours after the time of trauma, and was present in all TBI cases with death more than 72 hours later, thus there was a highly statistically significant positive correlation between the severity of reactive gliosis and survival time and a moderately positive correlation between the severity of gliosis and the severity of cerebral edema.

	Cerebral edema	Reactive astrogliosis
Cerebral edema	1	,565**
Reactive astrogliosis	,565**	1

Table 7.8 Correlation of association between lesion level cerebral edema severity and reactive gliosis severity. The correlation is significant at 0.01

Analysis of GFAP density and intensity in the fragments studied shows that there is direct positive, statistically significant correlations between changes in GFAP expression intensity and changes in GFAP+ astrocyte density in the fragments examined, with expression intensity being directly influenced by the increase in the number of reactive astrocytic cells, both in the lesional fragment and in the fragment contralateral to the lesion and in the hippocampus (Table 7.16.).

It was observed that there is a significant difference in the severity of reactive astrocytosis in the TBI cases compared to the control group in which we did not find changes in GFAP immunopositivity, these were only evident in the cases of the trauma group. Thus using immunohistochemical analysis of GFAP reaction we observed slight changes in intensity and cellularity of GFAP+ astrocytes even in the traumatic brain injury with immediate death in which we found weak GFAP expression with a slight increase in astrocytic cytoplasm adjacent to hemorrhagic foci.

One hour after the time of trauma we observed an increase in GFAP expression intensity, diffuse, both in the astrocytic body and in the astrocytic extensions, associated with a slight increase in cell density. In this case, low GFAP expression was identified in the hippocampus, and it was the only case in the acute TBI group with death in the acute period in which changes were found in hippocampal astrocytes.

In the group of TBI with sub-acute death, a weak GFAP intensity associated with a decrease in the number of reactive astrocytes was observed, but without a constant, linear evolution in relation to survival time. A decrease in the number of reactive cells and weak GFAP expression was also observed in the TCC group with survival longer than 72 hours, on day 8 and 9 from the time of trauma intense immunopositivity is observed.

A low GFAP density was observed more frequently in that lesional focus that showed subarachnoid hemorrhage, for which however statistical analysis of GFAP expression did not show a correlation between expression intensity and this type of hemorrhage.

In the present study we found a moderately positive correlation between GFAP expression intensity in astrocytes in the lesional focus and GFAP intensity in astrocytes in the contralateral focus. A weakly positive correlation was observed between the intensity of expression at the lesion level and GFAP intensity at the hippocampus level, while a moderately positive correlation was observed between astrocytic changes at the hippocampus level and those at the contralateral foci level.

Weakly positive correlations were also found between GFAP+ astrocyte density at the lesion level and astrocyte density at the level of the fragment harvested from the contralateral emisfer.

These observations suggest that GFAP is an indicator of the rapid astrocytic response following traumatic brain injury, not only at the level of the traumatic lesion but also at the level of the whole brain tissue.

Intense increased expression of neurofilament was identified in the TBI group with death more than 2 hours after the trauma, both at the level of the brain contusion fragment and at the level of the contralateral fragment, in which neurofilament immunopositivity with focal distribution of moderate intensity predominantly in the cortical layers was observed.

Changes in the intensity of anti-NF expression in the hippocampus we found in the case with survival of about 30 min, where expression of moderate intensity was predominantly in the soma of CA4 neurons and weak expression in the CA1 and subiculum region.
Positive anti-NF reactions in the soma and axonal extensions could be observed 18 hours after the trauma, present in perilesional arranged neurons.

The most frequent changes in immunoreactivity of Anti-neurofilament staining were observed in layers 3-5 and less frequently in layer 6. We did not identify changes at layer 1.

Positive β-APP expression was identified earliest at 30 min survival from the time of trauma, both at the level of the brain contusion zone and in the contralateral fragment, predominantly in the soma of neurons in layers 5-6. Also at 30 min, we identified positive β-APP expression in neurons in the CA4, CA1 and subiculum region.

In a single case of TBI with death 15 days after the time of trauma, we identified 3 patterns of β-APP expression distribution, linear - in axonal extensions of neurons in layers 5 to 4, retiform in layers 3-4 and focal in layers 1-2. In cases with survival longer than 16 days β-APP expression was fibrillary, non-specific.

STUDY 3: CASE STUDY: DETERMINATION OF TRAUMATIC INJURY MECHANISM BY CRANIAL RECOMPOSITION

One of the most important objectives of forensic examination of bone fragments with massive destruction is to determine the cause of death and the existence of traumatic injuries prior to destruction, and the cephalic extremity is one of the most prone regions to suffer aggression trauma and is also the region that undergoes the most burn-specific changes [20]. The most common method of attempted destruction of the body is by burning the building where the crime was committed or by burning the body using flammable substances. In the case of charred bodies, the destructive changes caused by fire make it difficult not only to identify the victim but also to identify the injuries causing death.

This study presents a case of homicide followed by burning of the body and the crime scene. At the time of discovery, the body was severely charred, with the cranial cavity containing numerous bone fragments of various sizes in both the neurocranium and viscerocranium with damage caused by the flames. On some of the fragments the presence of soft tissues with destructive changes was observed. Examination of the bone fragments revealed the presence of

a small fragment with a semicircular area of indentation on the outer plate and irregularly arranged cracks on the inner plate.

Given the appearance of the bone fragment suggesting the existence of a bone fracture by impaction, it was considered useful to reconstruct the skull in order to locate it.

For the reconstruction of the skull and viscerocranium, the method chosen was to reconstruct according to the anatomical landmarks of each fragment by "gluing" the fragments onto a "center" of modelling plasticine. In this way, the skull and viscerocranium were reconstructed in their quasi-totality, which allowed the reconstruction of numerous fracture lines and a fracture focus with infusion of quasi-circular shape, located on the left parietal side with a diameter of about 3 cm.

By this method it was possible to identify numerous fracture tracts located both in the viscerocranium and the neurocranium, but most importantly for the forensic expertise and for the judicial investigation it was possible to identify a fracture site located at the left parietal level, round-oval in shape with indentation and embossment, 3 cm in diameter, radiating from which radiates fracture tracts arranged meridional and equatorial, suggesting a strong impact with a relatively circular or round-edged body, 3-5 cm in diameter. At the same time, adjacent fracture paths, some unrelated to the main focus, suggest repeated striking of the cephalic extremity.

The case presented shows the importance of knowing the bone changes and the aspects of morphological aspects, caused both by the mechanical action of a traumatic agent and by exposure to flames. It also shows the importance of cranial reconstruction in the forensic examination of cranial bone fragments, in order to objectify the cause of death, especially in the absence of brain tissue.

Cranial reconstruction allows the identification and differentiation of ante-mortem lesional aspects from heat-induced bone changes. At the same time, cranial reconstruction can reveal radiating fractures and their convergence, which cannot be identified by examining bone fragments in isolation.

In this case cranial reconstruction allowed not only the identification of the traumatic lesion and its location, but also to identify the traumatic agent, whose characteristics were suggested by the morphology of the fracture at the parietal level. The large number of bone fragments was mainly due to the aggression committed with great violence and not only to the action of fire. In addition, the results of the histopathological examination support the importance of carrying out

and corroborating it with the macroscopic necropsy in the objective scientific proof of the cause of death.

CONCLUSIONS

Following the research, I carried out I was able to formulate the following conclusions

1. Traumatic brain injury has the highest incidence among people aged 61-70 years, the ratio of males: females suffering traumatic brain injury causing death is 3:1, the male population being representative for all age groups studied.
2. Approximately 13% of TBIs die immediately post-trauma and 60% of fatal TBIs are classified at the time of first clinical evaluation as severe TBI, with an average survival time of 9 days, with more than 50% of hospitalized TBI cases surviving between 2 and 10 days.
3. The most common circumstances of brain injury are falls from the same level and road traffic accidents, with road traffic accidents being the main circumstance of TBI in people up to the age of 50, while falls were the most common circumstance of TBI in people over the age of 60.
4. The most common brain lesion at autopsy is subarachnoid hemorrhage, the least common being traumatic intraparenchymal hematoma. 75% of autopsied TBI cases present with cranial fracture, with irradiated linear vault fracture of the skull base diagnosed in more than 40% of cases.
5. On routine histopathological examination, cerebral oedema is most commonly diagnosed, with 45% of cases analyzed showing severe oedema.
6. Comparative analysis of necropsy findings with imaging findings shows that on average in about 17% of cases there are discrepancies between ante-mortem and post-mortem diagnoses, with cerebral oedema being diagnosed differently most frequently. In 15% of the cases, imaging examinations missed the diagnosis of skull fractures.
7. Advanced hypoxic changes - small, eosinophilic neurons with hyperchromatic nuclei could be identified at the earliest 30 minutes after the time of trauma, red neurons were identified at the earliest 45 minutes in the cortex of the subarachnoid hemorrhage. In the hippocampus, changes following hypoxia were observed earliest in death at 30 minutes after trauma.
8. GFAP is a sensitive and specific indicator of the rapid response of astrocytes in brain injury, the reactive response of astrocytes at the level of the lesion has been demonstrated by GFAP antibody labelling in all cases of TBI, most commonly, GFAP expression being lost in

hemorrhagic areas, perilesional increased with associated changes in cell morphology and cell density.

9. In terms of GFAP expression in relation to survival time, changes in intensity are observed even in immediate post-traumatic death, in the lesional focus, while in the contralateral focus and in the hippocampus, they were observed at 30 minutes post-trauma. At the lesion level, increased GFAP expression intensity was observed in all groups belonging to the TBI group, while at the tissue fragment level in the counter-injury focus, it was identified only in the late death TBI group.

10. Neuronal damage was targeted by anti-neurofilament staining, it was overexpressed in neurons with hypoxia-induced changes in perihemorrhagic tissue and in neurons located in cortical layers 3-5 and less frequently in layer 6., at 4 hours NF deposits were observed in the neuronal body and at 18 hours accumulation of neurofilament in axonal extensions in the cerebral cortex.

11. Positive expression of β-APP in the neuronal body was identified in supra-acute, perilesional lesions, at the earliest 30 minutes after trauma. In axons, positive β-APP response is evident 4 hours after trauma. Intense immunoreactivity was observed up to 18 hours post-trauma, later expression is moderate or weak.

12. Regarding their usefulness in dating traumatic injuries, given the results of the study in which uneven intensity was observed in relation to survival time, I believe that such an interpretation should be made by concomitant investigation, correlating them with macroscopic aspects, clinical data, and investigation data.

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