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Evaluation of brain injuries in children deceased due to head trauma

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Abstract

Traumatic brain injuries (TBIs) present an ever-growing prevalence, especially in the developing countries. Although 80–95% are mild to average injuries, they determine multiple severe neurological sequelae and disabilities. Most of these injuries are caused by traffic accidents. We studied a number of 29 cases of severe TBIs, in children who deceased immediately or after a few days of survival. Most of them (over 68%) were caused by traffic accidents. The incidence of traumas increased by age, most cases being recorded in the age group 10–15 years old. The TBIs were complex ones. In 86.21% of the cases, the forensic examination highlighted the presence of cranial fractures; in 93% of the cases, there were highlighted complex meningo-cerebral injuries: leptomeningeal hemorrhage associated with brain contusion injuries and with intraventricular blood flood, as well as destructive lesions of brain dilaceration; only in 7% of the cases there were highlighted meningeal lesions, with no brain lesions. The severity of the brain injuries was quite varied, according to the force of the cause agent. The histopathological and immunohistochemical examinations showed that the severity of TBIs increased according to the survival time, by adding secondary lesions caused by brain ischemia and local inflammatory reaction.

Keywords: traumatic brain injury, child health, pediatrics, neuroinflammation, head injury.

Introduction

Traumatic brain injury (TBI) continues to affect millions of individuals all over the world. TBIs represent the main cause of death and disabilities, regardless the age of patients [1–3]. Every year, worldwidely, approximately 10 million people suffer a TBI [4, 5]; although 80–95% are mild to average injuries, they may determine multiple severe neurological sequelae and disabilities [6]. Only in the USA, every year there are recorded about 1.7 million patients with TBIs, and approximately 5.3 million people live with disabilities caused by a TBI [7, 8]. Of the 1.7 million TBIs diagnosed every year in the USA, approximately 30% (511 000 TBIs) are recorded in children aged between 0–14 years old, of which 35 000 require hospitalization, and over 2000 decease [9]. Due to the development of the car traffic and the increase of economic activity, there is estimated that, until 2020, all over the world, TBIs will become the third greatest healthcare problem, with enormous financial and social costs [4].

According to some opinions, TBIs in children are more severe due to a greater ratio between the cephalic extremity and the rest of the body, in comparison to adult individuals [10]. Most traumatic injuries involving children are caused by car traffic. *World Health Organization* (WHO), based on numerous studies, reported that the mortality caused by car accidents in children is higher in developing countries [11, 12]. Worldwidely, deaths caused by car accidents in children represent a prevalence of 10.7 in 100 000 inhabitants [10, 13].

Our study performed an analysis of the death cases caused by TBIs in 29 children aged less than 18 years old, undergoing autopsy within the Forensic Institute of Craiova, Romania, between 2011–2016, associated with studies of microscopy and immunohistochemistry for a complete and accurate histopathological diagnosis.

Materials and Methods

Our study is a retrospective one, regarding the deaths by TBI in underaged individuals between 2011–2016, in Dolj County, Romania. The data were extracted from the forensic reports on bodies, made within the Forensic Institute of Craiova, between 2011–2016, from the hospital observation sheets (when there existed any survival and hospitalization period), from the medical care records within the Emergency Room (ER) Unit (UPU) of the

Emergency County Hospital of Craiova, from investigation reports annexed to expertises, photographs and results of necropsy and pathological examinations requested at autopsies. The data were processed statistically, being performed charts for reporting the results. Also, there are provided figures and images on the main TBIs found in the studied period in underaged individuals.

For the histopathological study of brain injuries, there were harvested brain fragments from the injured and perilesional areas that were fixed in 10% neutral formalin solution and included in paraffin, according to the usual histological procedure.

The sectioning of the biological material was performed in the Microm HM350 rotary microtome, equipped with a section transfer system on water bath (STS, microM) and a Peltier cooling system of paraffin blocks. For the histological study, there were performed 4-μm thick sections, which were stained with Hematoxylin–Eosin (HE) and green light trichrome, the Goldner–Szekely (GS) technique.

For the immunohistochemical (IHC) study, the histological sections were collected on poly-L-lysine covered slides, for increasing the adherence of the biological material on the port-object slide, and dried, afterwards, in a thermostat, at 37^0C, for 24 hours. Then, the sections were deparaffinized and hydrated. For antigen demasking, the slides were boiled in a citrate sodium solution, pH 6, for 21 minutes (seven cycles of three minutes), in a microwave oven. The blocking of endogenous peroxidase was made by incubating the slides into 3% hydrogen peroxide, for 30 minutes, at room temperature, followed by a washing in distilled water for 10 minutes and a washing in 1% phosphate-buffered saline (PBS), for five minutes. After this, there followed the blocking of non-specific sites, by using 2% skimmed milk, for 30 minutes. The sections were then incubated with primary antibodies, for 18 hours (overnight), in a refrigerator, at 4^0C. The next day, there was applied the secondary biotinylated antibody for 30 minutes, at room temperature, followed by the washing in 1% PBS (three baths of five minutes), followed by the application of Streptavidin–Horseradish peroxidase (HRP) for 30 minutes, at room temperature, followed by blade washing in 1% PBS 3×5 minutes. The signal was detected by using 3,3'-Diaminobenzidine (DAB) (Dako) and the reaction was stopped in 1% PBS. There followed the Mayer's Hematoxylin contrasting, alcohol dehydration, xylene clarification and slide fixing by using a DPX environment (Fluka).

For the IHC study, we used the following antibodies: anti-CD68 (clone KP1, 1/100 dilution, Dako) for highlighting the macrophage reaction, including brain microglia; anti-glial fibrillary acidic protein (GFAP) (clone ab7260, 1/150 dilution, Abcam) for highlighting the microglia reaction.

Results

Within the Forensic Institute of Craiova, between 2011–2016, there were performed 3942 forensic autopsies; of these, 2414 cases suffered a violent death. Of TBIs, 29 cases, representing 1.2% of all the violent death cases, were recorded in individuals less than 18 of age.

The study of TBIs, according to age and type of aggression, showed the following (Figures 1 and 2):

- In babies (age group 0–1 years old), there were recorded two cases, both caused by car accidents, the babies being inside cars involved in collisions. The cars were not equipped with special protection chairs for this aged, imposed by the law.
- In toddlers aged between 1 and 5 years old, there were recorded eight cases of TBIs. Of these, four were caused by car accidents, two cases were caused by animal aggressions and two cases were caused by home accidents.
- In the age group 5–10 years old, there were recorded six cases of TBIs. Of these, three were caused by car accidents, a case was the result of a train accident, a case was caused by animal aggression and a case was caused by head injury with a hard body.
- In the age group 10–15 years old, there were recorded nine cases. Of these, seven were caused by car accidents, one case because of human aggression (injury with a hard body) and a case was caused by falling from height (falling from a tree).
- In the age group 15–18 years old, there were four cases of traumatic head injury, all caused by car accidents.

There was observed that of the 29 deaths by traumatic head injury, 20 (68.96%) were the result of car accidents.

Regarding the gender distribution of cases, in our study, we observed that 19 (65.51%) children were males and only 10 (34.48%) were females (Figure 3). Also, the TBIs in children according to the social environment showed significant differences. Thus, in the urban area there were recorded eight (27.59%) deaths, while in the rural area, there were recorded 21 (72.41%) deaths (Figure 4).

By analyzing the time passed from the injury until the individual's death, there was observed that in 12 (41.38%) cases, the injuries were quite serious, death occurring immediately. In five cases, there was a survival period less than 24 hours; three children had a survival period between one and five days; six children had a survival period between 5–10 days, and only three children survived over 10 days (the data was found in the clinical observation sheets annexed to the forensic expertise reports) (Figure 5).

Forensic examination

In 25 of 29 (86.21%) cases, there were highlighted fractures of the skull bones; however, only in three cases there was observed the presence of an extradural collection, an aspect that may be explained by a strong adherence of dura-mater to the endocranium, in children. In 27 (93%) cases, there were observed complex meningo-cerebral injuries, the leptomeningeal hemorrhage being associated with brain contusion injuries and an intra-ventricular blood flood, more or less abundant, and brain dilacerations. Only in two cases there were observed meningeal injuries without visible brain lesions. In 14 cases, there were observed important dilaceration brain injuries in the contusive foci (Figure 6).

By studying the morphology of skull fractures, there were described both direct fractures of the cranial vault, without base irradiation, and fractures with a vault impact point and with an irradiation at skull base (indirect base fractures). The morphological aspect of the fractures varied from simple linear fractures or depressed fractures, until aspects of complex multieschilous fracture, with a detaching of bone eschilas and brain matter hernia

(Figure 7, a and b). There were not identified any direct fractures with skull base impact point, all the fractures concerning the base being irradiated from the vault or mediated fractures due to spine bones or mandible.

In 27 cases, there were highlighted various degrees of brain contusion. There were found minor contusion injuries, and also serious brain contusions with brain dilaceration in the contusive foci and cerebral trunk contusions. The localization of contusions in relation to the brain cortex was both cortical and subcortical (Figure 8). The destructive injuries of brain dilaceration were found in the contusive foci, as counterpart or direct impact injuries; there were found various aspects, from extended dilacerations in both brain hemispheres (Figure 9), to localized dilaceration foci.

Leptomeningeal hemorrhages became localized or diffuse injuries (Figure 10).

The intraventricular blood flood was present in most cases, being highlighted either in the lateral ventricles (in the Virchow sections), in the ventricles III and IV or in the Sylvius aqueduct (Figure 11).

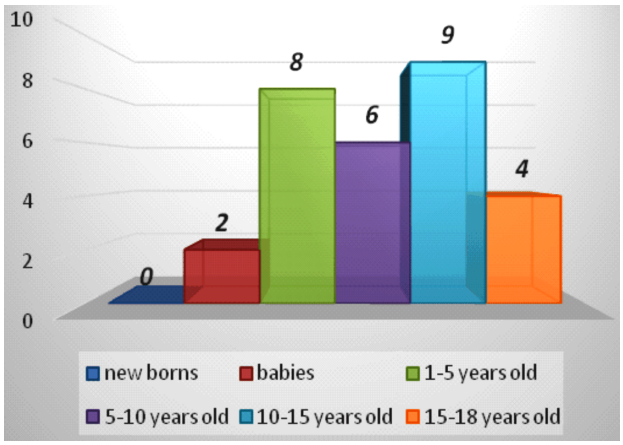

Figure 1 – ***Distribution of TBI cases according to age. TBI: Traumatic brain injury.***

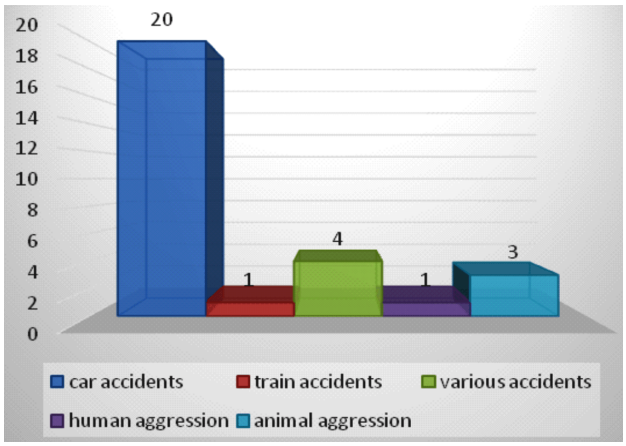

Figure 2 – ***Case distribution according to legal framing.***

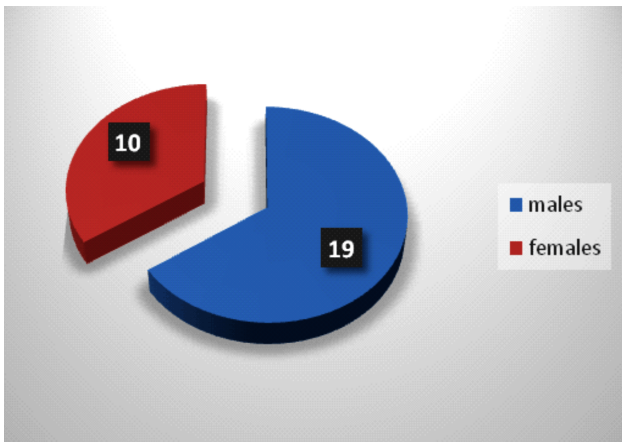

Figure 3 – ***Distribution of TBI cases according to gender. TBI: Traumatic brain injury.***

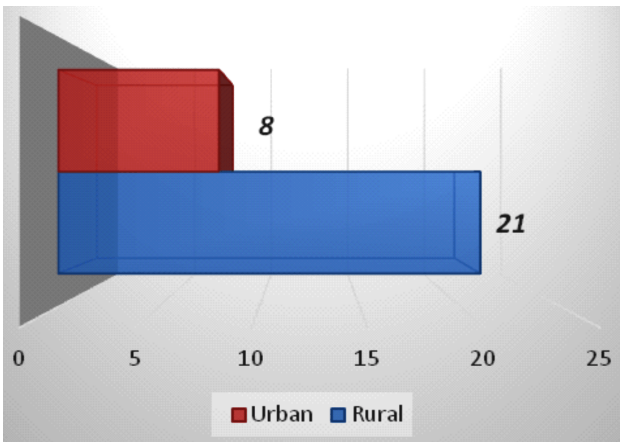

Figure 4 – ***Case distribution by TBI death in underaged individuals according the living environment. TBI: Traumatic brain injury.***

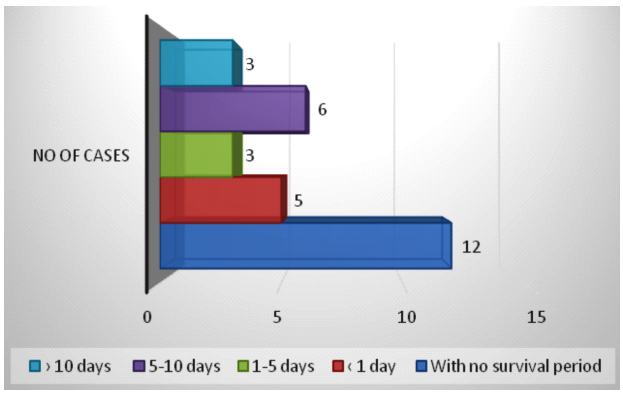

Figure 5 – ***Distribution of cases according to the survival period.***

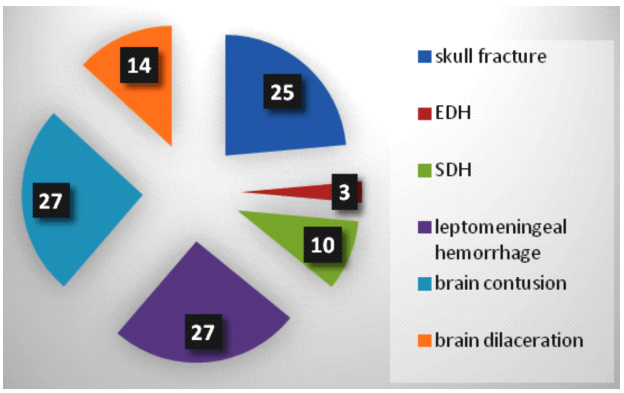

Figure 6 – ***Morphological macroscopic distribution of traumatic brain injuries. EDH: Extradural hematoma; SDH: Subdural hematoma.***

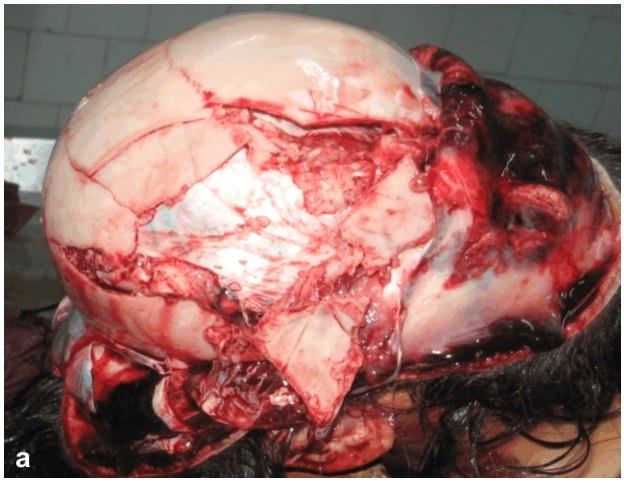

Figure 7 – ***(a) Image of multieschilous fracture with eschilas detaching, dural disruption and subadjacent brain dilacerations in a 10-year-old girl, caused by a car accident; (b) Trajectory of irradiated fracture (same case).***

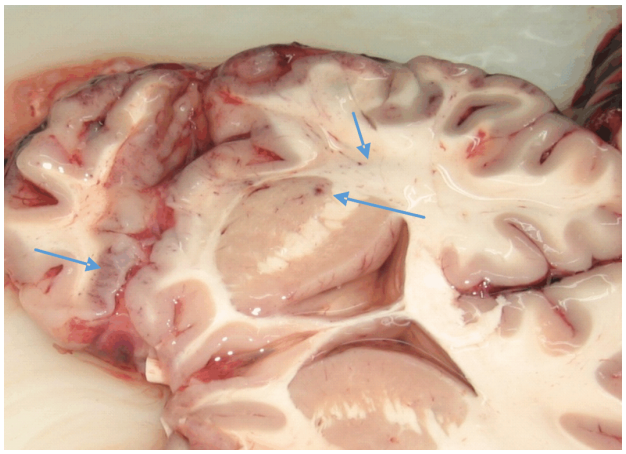

Figure 8 – ***Diffuse contusions localized cortically and subcortically (car accident).***

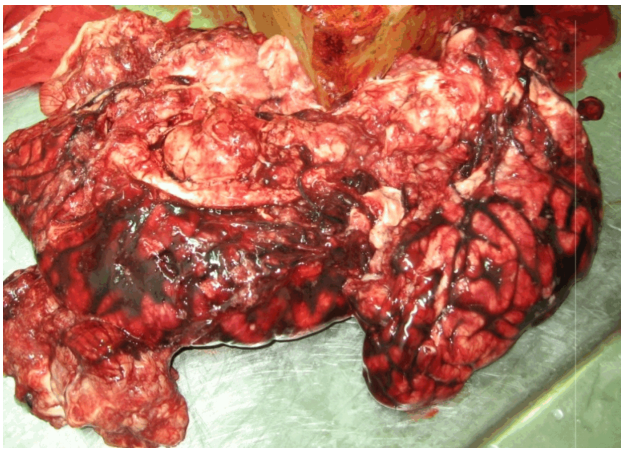

Figure 9 – ***Image of massive dilacerations that affected both hemispheres, in a 2-year-old boy (animal aggression).***

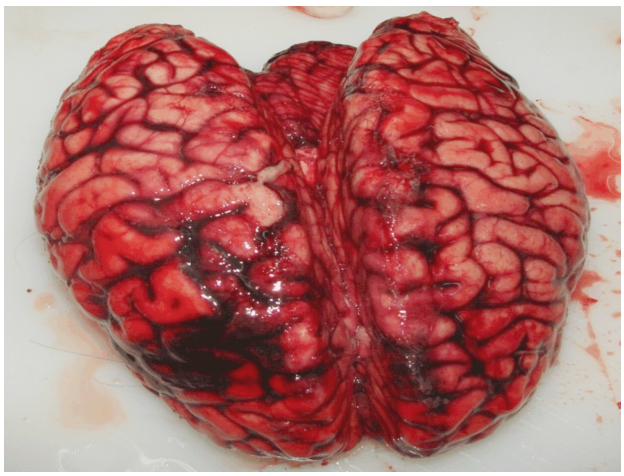

Figure 10 – ***Diffuse leptomeningeal hemorrhage in an 11-year-old girl (human aggression).***

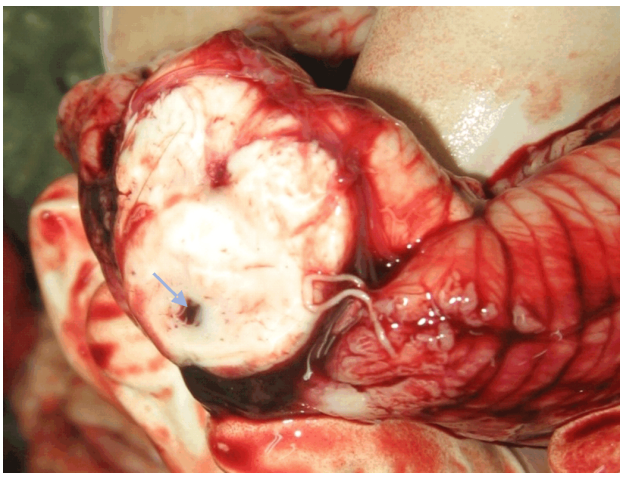

Figure 11 – ***Intraventricular blood flood, in the Sylvius aqueduct, associated with brain trunk contusions, present in a 2-year-old boy (car accident).***

Histological and IHC study

The histological and IHC study took into consideration the highlighting of the pathological injuries of the encephalon in the traumatic foci, and also around them, and the correlation of the injury severity with the patients' survival time, as in primary injuries caused by traumatic force there are added secondary injuries caused by the local inflammatory process and by the changes of brain microvascularization secondary to the traumatic injury.

In the patients in whom death occurred immediately after the traumatic injury, the microscopic changes of the encephalon, outside the brain contusion foci, were minor

ones, the secondary reaction being absent (Figures 12 and 13). There was observed the presence of diffuse hemorrhages in the leptomeninges and the presence of a moderate perineuronal edema. There were not highlighted any changes of the microglias or macroglias.

After four days since the traumatic injury, the histopathological changes were more serious; the perineuronal and perivascular edemas were more visible, part of the cortex neurons presented vacuolizations or cytoplasmic condensations, nuclear pyknosis and karyolysis, phenomena of neuronal apoptosis and autolysis, with the formation of "neuronal phantoms". The neuropile appeared disorganized, mildly granular, with a mild perifibrillary edema, showing the presence of some lesions in the dendrites and axons (Figures 14 and 15). In the nervous parenchyma, there was observed the activation of larger macroglias, with large, hypochrome nucleus and long, thick extensions and with an intense reaction to the anti-GFAP antibody (active glyosis) (Figure 16). Also, there was observed the presence of perivascular macrophages, with a rich, vacuolar cytoplasm and intense reaction to the anti-CD68 antibody (Figure 17).

Seven days after the traumatic injury, the histopathological lesions close to the brain foci contusion were more highlighted. The brain parenchyma presented a spongy aspect by the death of neurons and glial cells, disorganization of neuronal extensions, presence of apoptotic bodies, perineuronal and perivascular edema (Figures 18 and 19). Also, there were identified isolated areas of intraparenchimatous microhemorrhages and perivascular hemorrhagic infiltrates through the Virchow–Robin spaces, in distant areas from the traumatic injury impact zone (Figures 20 and 21).

The reaction of macrophages was clear, being identified numerous activated intraparenchymatous microglias (Figure 22) and also numerous macrophages present in the perivascular hemorrhagic infiltrate (Figure 23). Also, the macroglias appeared more numerous, hypertrophied, with moniliform or fragmented extensions, with a clear shape and an intense reaction to the anti-GFAP antibody (Figure 24).

Nine days after the traumatic injury, the pathological changes of the brain were more intense in comparison to those previously observed. Neuronal death was quite intense in the areas next to the brain dilaceration foci, while far from the contusive foci, there were observed "red neurons" characteristic to neuronal hypoxia (Figures 25 and 26). The microglia reaction was, also, an intense one (Figure 27), as well as the reaction of the macrophage system cells (Figures 28 and 29). Sometimes, there were highlighted microhemorrhagic foci and even vascular thromboses (Figure 30).

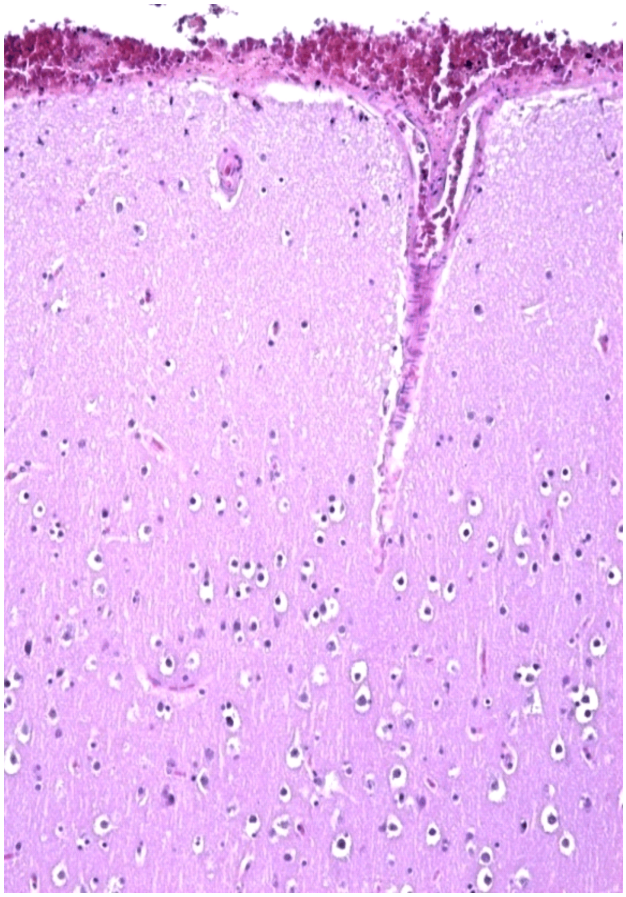

Figure 12 – ***Image of the cortex in the immediate proximity of the brain contusion foci, with leptomeningeal hemorrhage and a moderate perineuronal edema, in a patient in whom death occurred immediately after the traumatic injury (HE staining, ×100).***

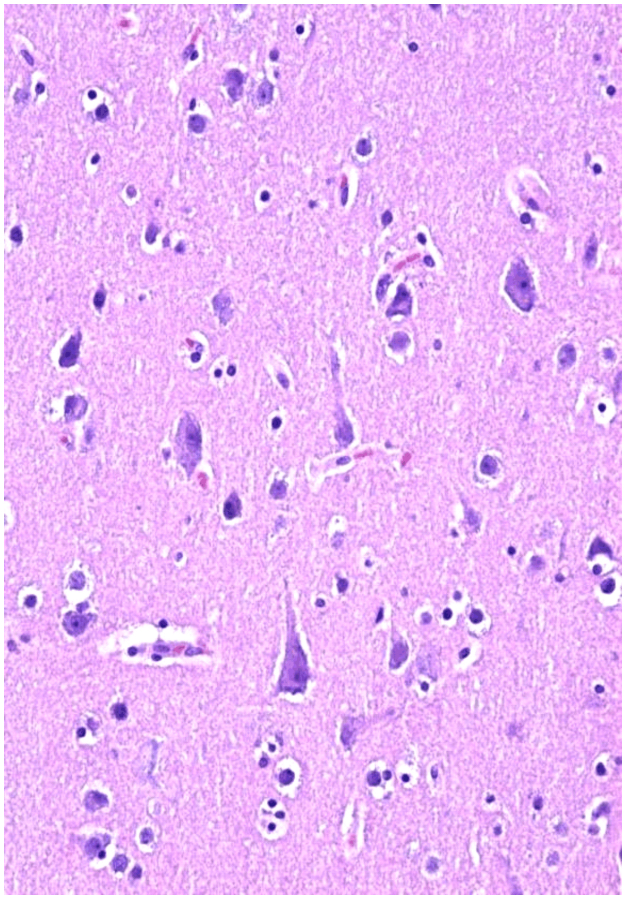

Figure 13 – ***Image of the cortex with no microscopic changes in the proximity of the contusive foci, in a child in whom death occurred immediately after the traumatic injury (HE staining, ×200).***

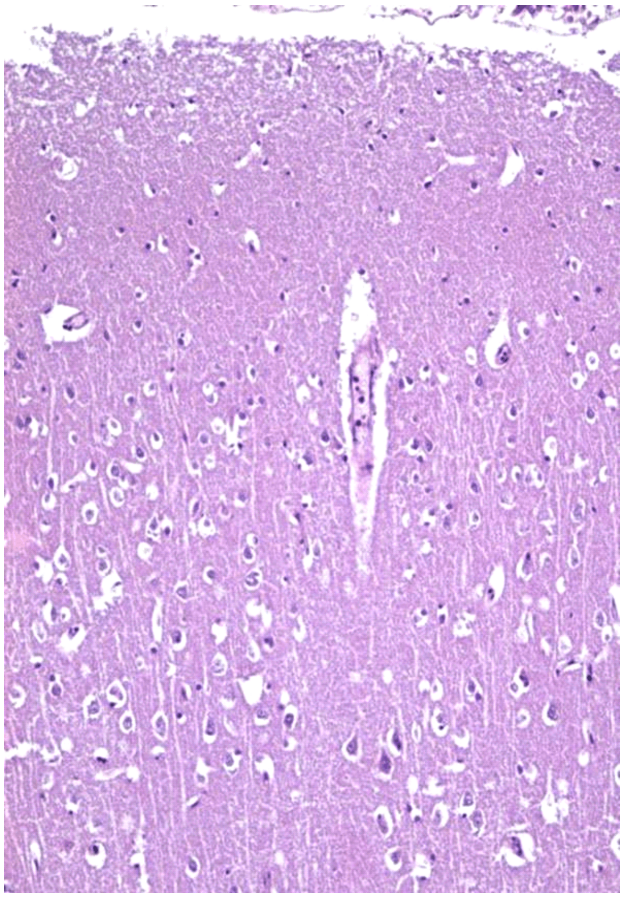

Figure 14 – ***Overall microscopic image of the brain cortex after four days since the traumatic injury. There may be observed the leptomeninges detachment, the presence of a perivascular and perineuronal edema, associated with apoptosis and neuronal autolysis processes (HE staining, ×100).***

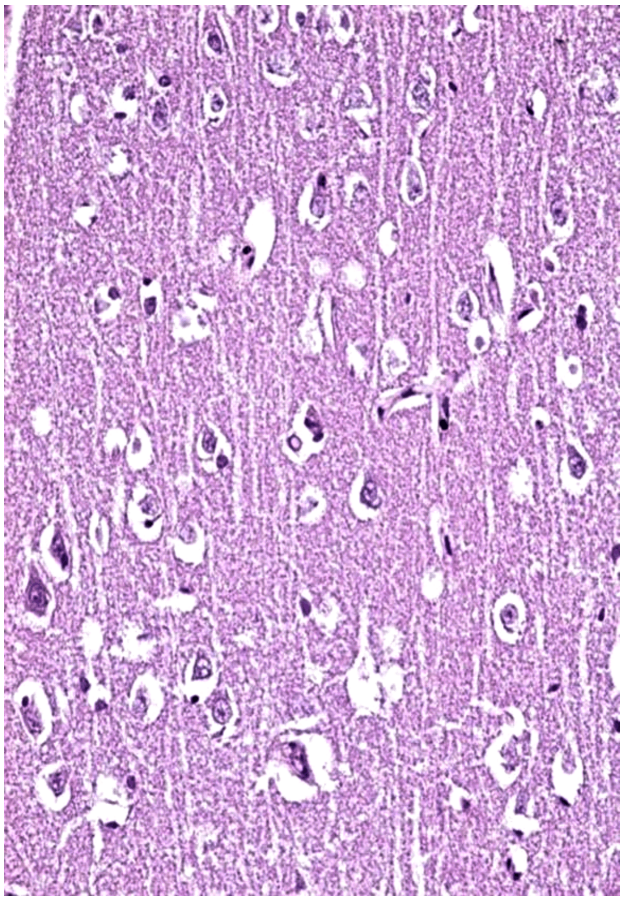

Figure 15 – ***Detail from the previous figure, where we can observe the deep alteration of the neurons and the neuropile, neuronal apoptosis and images of "neuronal phantoms" (HE staining, ×200).***

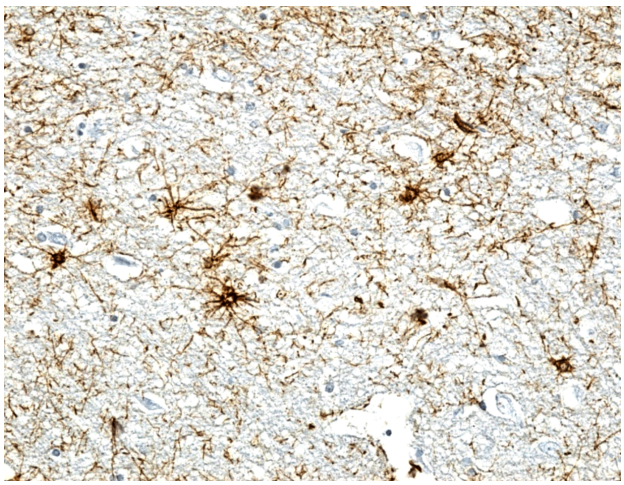

Figure 16 – ***Moderate reaction of macroglias after four days of survival (Anti-GFAP antibody immunomarking, ×100). GFAP: Glial fibrillary acidic protein.***

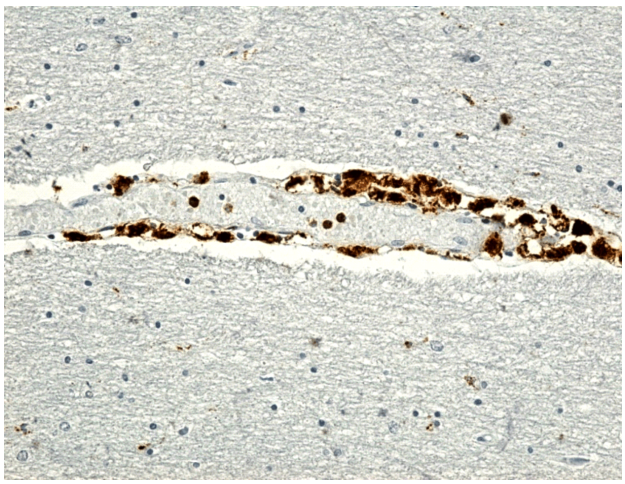

Figure 17 – ***Perivascular infiltrate with numerous macrophages, after four days of survival (Anti-CD68 antibody immunomarking, ×200).***

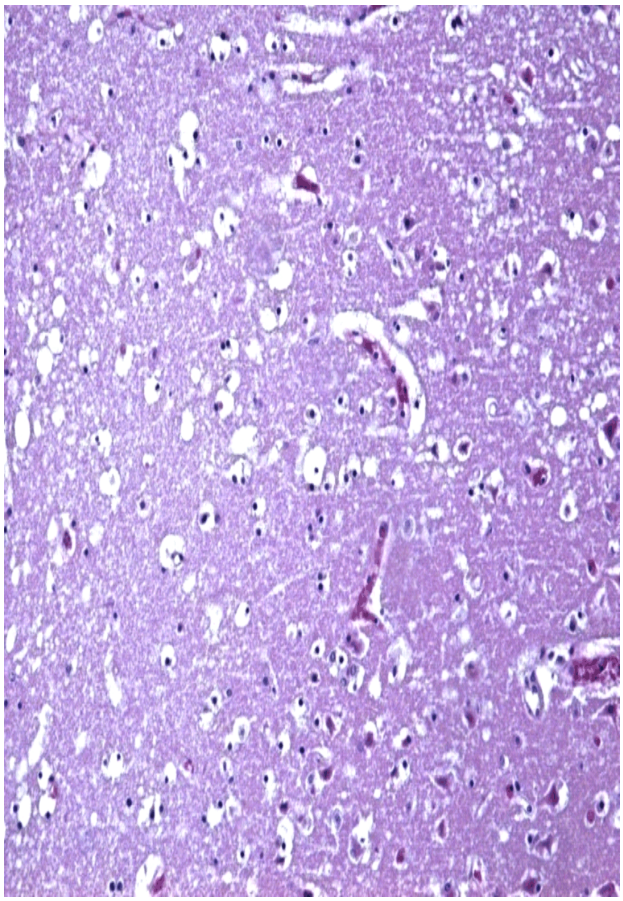

Figure 18 – ***Brain parenchyma with a spongy aspect caused by the death of neurons and glial cells (HE staining, ×100).***

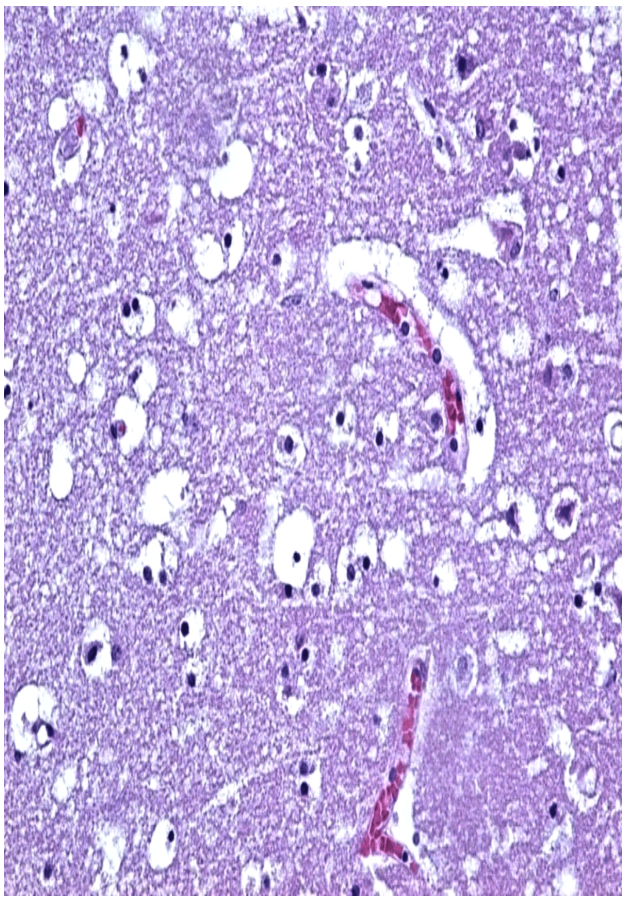

Figure 19 – ***Detail from the previous image, where we can observe the presence of apoptotic bodies, as a result of neuronal and glial death, perivascular and peri-neuronal edema and the deterioration of the neuropile as a result of the structural changes and neuronal extensions (HE staining, ×200).***

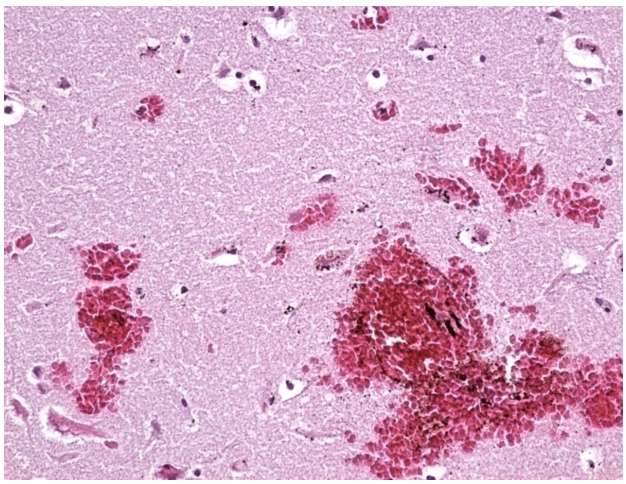

Figure 20 – ***Microhemorrhages in the white matter, distant from the contusion foci (HE staining, ×200).***

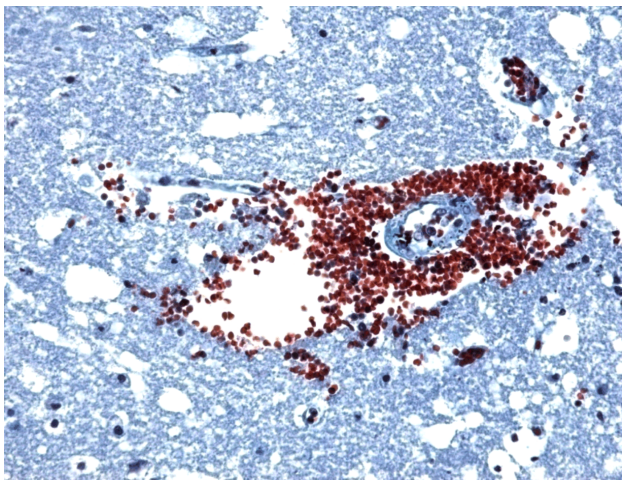

Figure 21 – ***Perivascular microhemorrhages through the Virchow–Robin space (GS trichrome staining, ×200).***

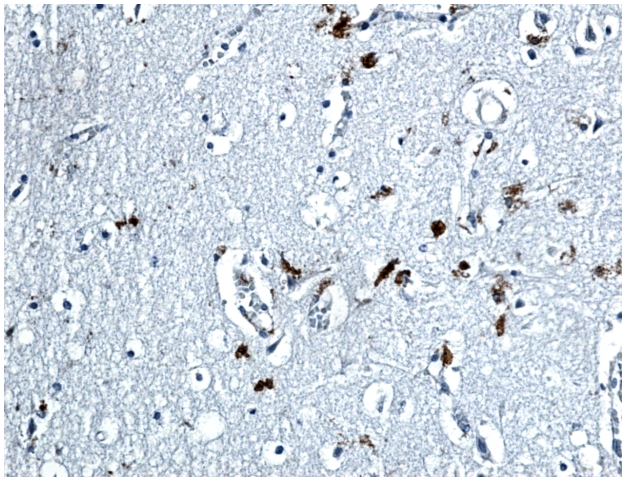

Figure 22 – ***Brain parenchyma with numerous activated microglias (Anti-CD68 antibody immunomarking, ×200).***

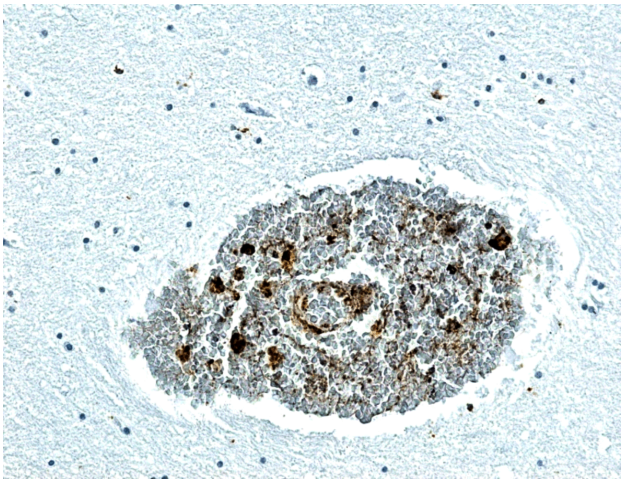

Figure 23 – ***Numerous macrophages present in the perivascular hemorrhagic infiltrate in the Virchow–Robin space (GS trichrome staining, ×200).***

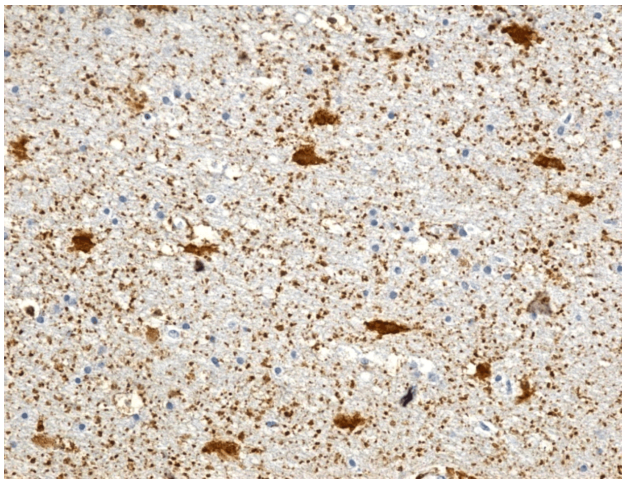

Figure 24 – ***Hypertrophied macroglias, with amputated extensions, fragmented and with an uneven shape (Anti-GFAP antibody immunomarking, ×200). GFAP: Glial fibrillary acidic protein.***

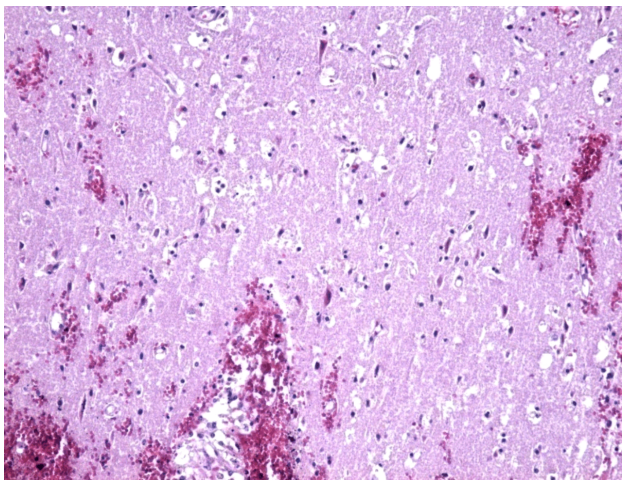

Figure 25 – ***Brain cortex with microhemorrhage foci and intense phenomena of neuronal apoptosis (HE staining, ×100).***

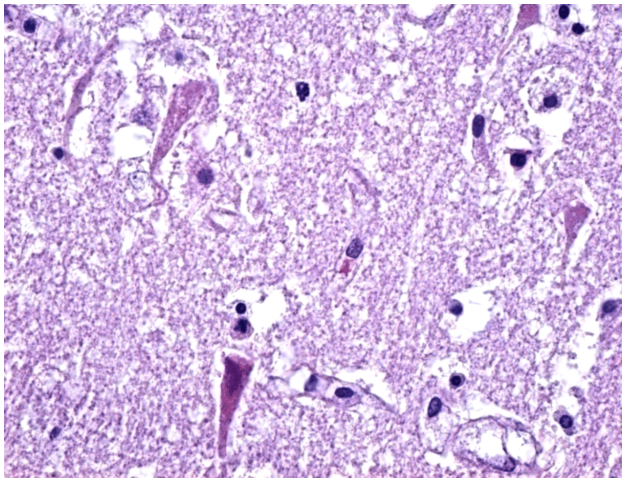

Figure 26 – ***Area of brain cortex distant from the contusive foci, with ischemic neurons (red neurons) and neuronal apoptosis (HE staining, ×400).***

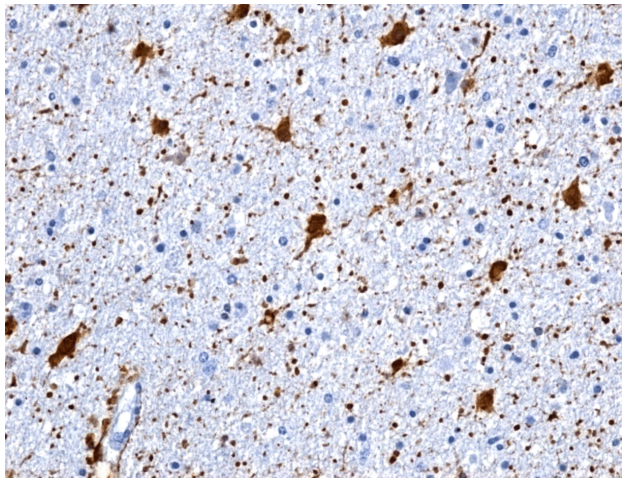

Figure 27 – ***Activated, hypertrophied macroglias, with amputated or fragmented extensions (Anti-GFAP antibody immunomarking, ×200). GFAP: Glial fibrillary acidic protein.***

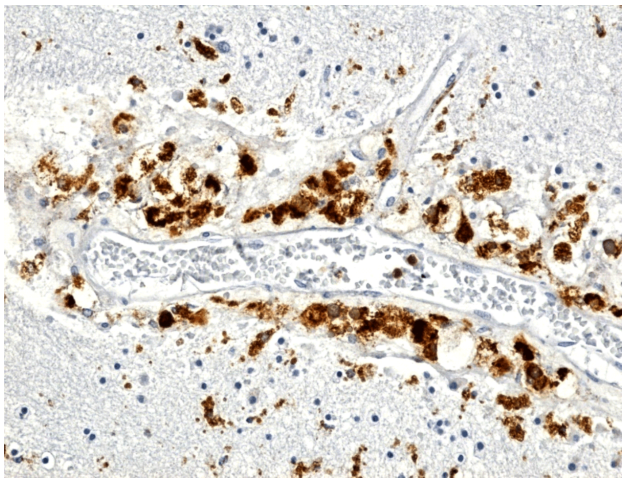

Figure 28 – ***Intense reaction, both perivascular and intra-parenchymatous, of microglias and macrophages (Anti-CD68 antibody immunomarking, ×200).***

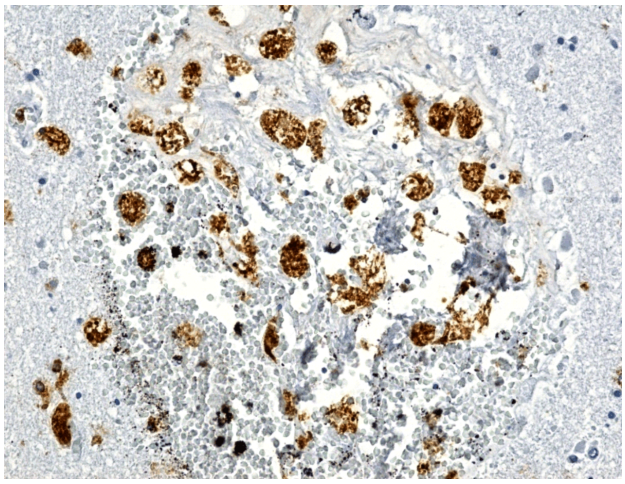

Figure 29 – ***Intense reaction of the macrophages in the hemorrhage and brain dilaceration foci (Anti-CD68 antibody immunomarking, ×200).***

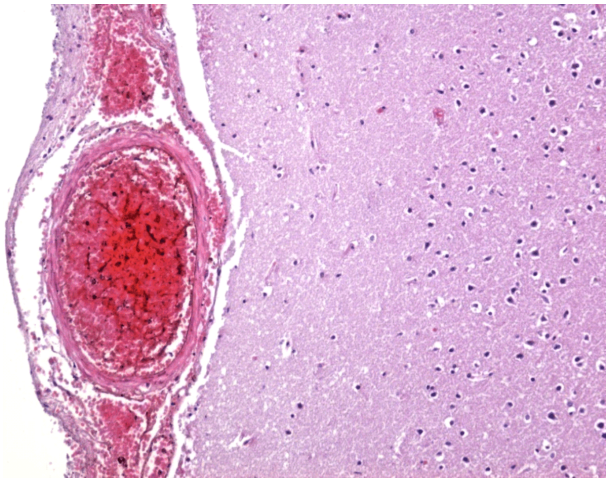

Figure 30 – ***Vascular thrombosis in the meninges (HE staining, ×100).***

Discussion

In the last decades, TBIs in children have become a major problem, threatening the children's health state, because they have devastating effects upon the child's brain and, moreover, they intensify the social and economic burden [14]. In this respect, there may be mentioned that in the USA, in 2000, there were hospitalized 50 658 children with TBI, aged less than 17 years old, and the hospitalization expenses exceeded 1 billion dollars [15]. Even mild TBIs may have severe consequences, as a child's injured brain cannot mature at the same time as the physical growth and development, which determines motor, cognitive, mental or memory disorders, making their social integration more difficult [16–18]. Some consecutive TBI disabilities may affect the individual for the rest of his/her life [19–21].

Of great concern should also be the fact that TBIs have a tendency to increase all over the world, alongside the development of car traffic. Only in the USA, between 2006 and 2016, the rate of children with TBI visiting the ER departments of hospitals was eight times higher than the total rate of ER visits due to other conditions [3].

Fortunately, the number of TBI cases resulting in the children's death is quite low. In our study, the death of children aged less than 18 years old represented only 1.2% of the total of violent deaths. Other studies also showed that, even though the global number of TBIs increased, and it is ever growing every year, there is a lower rate of deaths, yet a greater number of individuals live with disabilities caused by TBIs [22].

In our study, most deaths (68.96%) were caused by car accidents. Numerous other studies obtained similar results, showing that, almost worldwidely, car accidents represent the cause of the most frequent TBIs and death in children [1, 12, 23–26]. Most studies showed that, in the developing countries, there take place more severe car accidents that cause TBIs and death in children, due to the poor traffic infrastructure, the use of old cars or the lack of a preventive legislation. WHO also reported that death by car accidents in children is higher in low and average income countries, in comparison to developed countries [10].

Like other authors [27], as well, we found that most TBIs and deceased patients were boys. Also, in our study, most children deceased by TBIs came from the rural area (72.41%). In the USA, as well, some studies showed that in the rural area, the annual rate of the TBI incidence was 107/100 000 children, while in the metropolitan areas, the same rate was only of 71/100 000 children. Also, the mortality of TBI in children was higher in the rural area than in the urban area [28, 29]. In the rural area, it seems there are more risk factors than in the urban area. Moreover, it seems that, all over the world, there is a poorer medical assistance system in the rural area than in the urban area. These may be the main causes for the TBI incidence and mortality differences between the rural and urban areas [30–32].

The forensic study performed by us highlighted that only in two (6.9%) cases death occurred due to meningeal injuries, without any brain injuries; in the other 27 (93.1%) cases there were highlighted multiple injuries affecting, more or less, the encephalon nervous tissue. The skull fractures were identified in 25 (86.21%) cases, most of the fracture directions affecting more bones of the skull.

The intensity of meningo-cerebral injuries varied from one case to another, especially due to the mechanic forces acting upon every child's skull. Most studies showed that TBIs cause both extra-parenchymatous injuries (epidural hematomas, subdural hematomas, subarachnoid hemorrhage and intraventricular hemorrhage) and also intraparenchymatous injuries (intracerebral hemorrhage, brain contusions or dilacerations) [33, 34]. Some authors showed that skull fractures are quite frequent in TBI patients, being identified in 45–57% of the patients requiring hospitalization [35].

The histological and IHC studies performed by us tried to evaluate the pathological changes of the nervous tissue, according to the time passed from the traumatic injury to death, as it is well-known that the TBI physiopathology is a complex one and intricate both in primary and secondary injuries, caused by the vascular changes and the local inflammatory reaction [1]. The secondary reaction has the role of protecting the brain from other lesions and to contribute to the restoration of the nervous tissue and functional recovery, as well [36]. The secondary reaction may occur a few minutes or days since the primary impact and consists of a series of molecular, chemical and inflammatory changes that emphasize the neuron depolarization by releasing driving neurotransmitters (such as glutamate and aspartate), leading to the intracellular calcium increase. The intracellular calcium activates a series of mechanisms, with consequences involving the activation of caspases and formation of free radicals, which determine both a direct and an indirect cell degradation, through an apoptotic process. This degradation of neuronal cells is associated with an inflammatory response that continues to affect the neuronal cells and increases the permeability of the hematoencephalic barrier, thus generating brain edemas. After the second stage of emphasizing the neuronal injuries, there follows the recovery period, consisting in the anatomic, molecular and functional reorganization of the injured brain structures [1].

The microscopic study we performed showed that the neuronal injuries close to the contusive foci amplify with the survival time after TBI. In the subjects in whom death occurred in less than 24 hours after TBI, the neuronal injuries were minimal, being represented only by a minimal perineuronal edema. These lesions are mostly the result of the direct mechanic effect of the traumatic injury and/or of the inertia forces on the brain [28]. It is obvious that the traumatic agent, according to the mechanic force, may produce direct neuronal injuries, axon rupture and blood vessels lesions [37, 38]. At the same time with the direct mechanic effect, there is also triggered the secondary reaction, which may also lead to cell death when certain limits are exceeded [39, 40].

In our study, we observed that neuronal injuries and the activation of microglias and macroglias were intensified in the distant areas from the traumatic impact area, as the children's survival time increased. The perineuronal and perivascular edema were ever growing, neuronal death becoming more clear, and the glutamate and aspartate perivascular and intraparenchymatous macrophage number increased. Our study showed once more that TBIs affected the intimate relation between neurons, glial cells and blood vessels, thus altering the molecular mechanisms that maintain the homeostasis from the encephalic parenchyma [37, 41].

The blood vessel damaging in children deceased by TBI was manifested by leptomeningeal, intraparenchymatous or perivascular microhemorrhages and hemorrhages (through the Virchow–Robin spaces), which we believe determined the onset of a brain hypoxia state that contributed, in its turn, to the appearance of ischemic neurons (red neurons) and to neuronal death. In our opinion, brain hypoxia consecutive to the brain vascular network destruction, causes mitochondrial dysfunction in the neurons, which, in its turn, may lead to calcium cellular homeostasis disbalance, disbalance of membrane interchanges, neuronal depolarization, intra- and extraneuronal water accumulation, free radical generation, causing, by all these mechanisms, degenerative lesions of the neuronal bodies and extensions, followed by the occurrence of neuroinflammatory processes [42–44].

In our study, we observed that the inflammatory processes occurred in the brain after a few days since the traumatic aggression and they manifested mainly through the reaction of macroglias and the macrophage cells (microglias and macrophages). We consider that the main cells triggering the brain reaction are the astrocytes (macroglias) and the microglias. In their turn, these cells are activated by the changes of the local microenvironment because of the traumatic injuries [45–47]. After a few days since the traumatic injury, we observed an increase of the macroglia number and sizes (a process known as "reactive glyosis"), and also of their reactivity to the anti-GFAP antibody (a marker of intermediary filaments), both in the white matter and in the brain cortex. According to some studies, the glial activation induces morphological and functional changes that influence the interactions between the neurons and macroglias, thus causing dysfunctions of the neurons and synapses, disbalances of the neurotransmitters and even cell death [48].

The microglias represent approximately 15% up to 20% of the brain cells, they are cells of the immune system that actively participate in maintaining the brain homeostasis, and they also coordinate the inborn immune responses in order to fight the pathological processes in the brain [49].

As shown by us, in TBIs, the macroglias become rapidly activated, they increase in size, they retract their extensions and become round or ovalary, capable of mobilization and phagocytosis. They phagocyte the cell and tissue debris and stimulate the inflammatory response by the cytokine secretion, in order to initiate the tissue healing process [50, 51].

In our study, besides the activation of intra-parenchymatous microglias, we observed the presence of numerous active perivascular macrophages, in the blood infiltrates of the Virchow–Robin spaces, in the areas of intracerebral hemorrhage and even in the areas of brain dilaceration, which makes us think that these have their origin in the monocytes of blood vessels.

Conclusions

Between 2011–2016, in the Forensic Institute of Craiova, there were recorded 29 deaths in children less

than 18 years old, caused by TBIs. The most numerous (68.96%) were caused by traffic accidents. Most deaths (72.41%) were recorded in the rural area. Regarding the gender distribution of cases, we observed that 65.51% were recorded in males and only 34.48% in females. The most affected age was between 10–15 years old. The forensic examination highlighted the presence of complex fractures of the skull bones in 25 (86.21%) cases. In 27 (93%) cases there was observed the presence of some complex meningo-cerebral lesions, the leptomeningeal hemorrhage being associated to brain contusion lesions and with an intraventricular hemorrhage, more or less abundant, or brain dilacerations in contusion foci. Only in two cases there was observed the presence of meningeal lesions without any brain injuries. The pathological and IHC studies highlighted an increase of the neuronal lesions in the brain perilesional areas, simultaneous to the increase of the survival period of patients.

Conflict of interests

The authors declare that they have no conflict of interests.

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