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Diffuse Vascular Injury in Fatal Road Traffic Accident Victims: Its Relationship to Diffuse Axonal Injury*

ABSTRACT: The authors have reported a macro- and microscopic study of brain lesions in 120 victims of fatal road traffic accidents, independent of the survival time. Diffuse vascular injury (DVI) was found in 14 patients (11.7%). All patients with DVI died within 24 h after the accident. The 14 patients with DVI also showed severe (Grade 2 or 3) diffuse axonal injury (DAI). Since DVI is restricted to road traffic accidents and incompatible with life, the high frequency observed in our series could be explained by the fact that all 120 patients were victims of road traffic accidents, and 69.2% had died within 24 h after the accident. The association between DVI and severe DAI (Grades 2 and 3) suggests that both lesions depend on the same mechanism, with the degree of axonal and vascular damage being determined by the intensity of the head acceleration. Our results show a relationship between DVI and DAI that suggest there may be a spectrum or at least a continuum between these entities as distinct from DVI being a separate entity.

KEYWORDS: forensic science, forensic pathology, diffuse vascular injury, diffuse axonal injury, head trauma, road traffic accident

The pathological finding of multiple small hemorrhagic brain lesions of traumatic origin was first drawn attention to by Cassaca (1). Subsequently, cases of this pathology were reported only by Tomlinson (2), Strich (3), and Adams et al. (4), who named it diffuse vascular injury (DVI).

That DVI is not well known is due to its being essentially a pathological entity of little clinical importance, as it causes death in less than 24 h. Also, fatal head trauma victims are not usually subjected to a detailed pathological examination. However, a better knowledge of DVI is called for, since, as will be demonstrated, this type of lesion is relatively frequent in victims of severe head trauma caused by road traffic accidents.

As DVI and diffuse axonal injury (DAI) are lesions produced by acceleration of the head, and the axon injury occurs at lower acceleration levels than those required to cause vascular rupture (5), we raise the hypothesis that DVI should always be associated with DAI in road traffic accidents. The aim of the present study is to demonstrate that DVI is always associated with severe DAI in victims of fatal road traffic accidents.

Materials and Methods

One hundred and twenty victims of road traffic accidents, who had sustained either a motor vehicle accident (51 individuals) or an auto-pedestrian injury (69 individuals), autopsied in the period between 1989 and 1993 in Belo Horizonte, Minas Gerais, Brazil, were studied. Both the victims that had died after being admitted to the hospital and those whose death had occurred at the site of the accident or before admission were included. The autopsies were performed within 24 h of death. Complete postmortem examinations were performed in every case. After the trunk and limbs had been examined, the external lesions on the head and neck were described, followed by removal of the brain and description of the bone and intracranial lesions found. All brains had been fixed in 10% formalin solution for a minimum period of three weeks. After the external brain surface had been described, frontal sections through the cerebral hemispheres, horizontal sections through the brain stem, sagittal sections through the left cerebellar hemisphere, and oblique sections through the right cerebellar hemisphere were made. The sections were separated by 10-mm intervals at cerebral hemispheres level and by 5-mm intervals at brain stem and cerebellum levels.

Fragments for microscopic examination were taken from the frontal, parietal, temporal and occipital lobes, corpus callosum and fornix, basal ganglia, thalamus, hypothalamus, midbrain, pons, medulla, and cerebellar hemispheres. The fragments were processed for paraffin embedding, cut into 7-μm sections, and stained by hematoxylin-eosin. In addition, frontal sections of the brain, including parasagittal regions from both sides of the frontal lobes, basal ganglia, internal capsule, corpus callosum (at three levels), anterior commissure and fornix, and horizontal sections of the brain stem were also processed for paraffin embedding, cut into 7-μm sections, and stained for the identification of axons with a mouse monoclonal antibody to neurofilament proteins 70-, 160- and 210-kDa (Dianova-Immunotech, Hamburg, Germany) at a dilution of 1:200. The sections were incubated for 2 h at 4°C. For the visualization of the reaction product, the sections were reacted in 0.05% 3,3 diaminobenzidine tetrahydrochloride (Sigma Chemical Company, St. Louis, MO) using the peroxidase-antiperoxidase

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method (PAP). The histological sections were counterstained with hematoxylin. For positive controls, histological sections of normal brains were used. For negative controls, the phosphate buffer solution or normal mouse serum was used instead of the primary antibody.

DAI was graded according to the criteria proposed by Adams et al. (6): Grade 1—presence of axonal swellings and axonal bulbs; Grade 2—presence of hemorrhagic lesion in the corpus callosum; Grade 3—presence of primary hemorrhagic lesion in the dorsolateral quadrant of the rostral brain stem. In those patients that died instantaneously or before reaching hospital or that had died immediately after being admitted to the hospital, the presence of DAI was assigned by the finding of hemorrhagic lesion (macro- or microscopically) in the corpus callosum and/or in the dorsolateral quadrant of the rostral brain stem. Axonal injury was considered as evidence of DAI when axonal swellings and bulbs were diffusely distributed throughout the brain, although preferentially located in the corpus callosum, the internal capsule, the cerebral white matter, and the rostral brainstem (4,7). Axonal injury around focal lesions (hemorrhages, contusions, infarcts) was not considered as DAI.

DVI was identified as widespread, multiple periarterial, perivenous, or pericapillary hemorrhages in the cerebral white matter and/or in cerebellar white matter, cerebral cortex, basal ganglia, thalamus, and brain stem. Isolated or sparse focal hemorrhages, anywhere, were not considered as DVI. Also, focal hemorrhagic lesions characteristic of DAI Grades 2 and 3, respectively, in the corpus callosum (Fig. 1) and in the dorsolateral quadrant of the rostral brain stem (Fig. 2) or secondary to increased intracranial pressure (Duret's hemorrhage in the midline of the tegmentum of the midbrain and pons—Fig. 3) were not considered as DVI. Other possible causes of multiple small hemorrhages, e.g., fat emboli, were not found in the present series.

The state of consciousness on hospital admission was evaluated by the Glasgow coma scale (GCS) established by Teasdale and Jennett (8).

To evaluate the association between DVI and DAI, the qui-square test with Yates' correction or, where indicated, Fisher's exact test was used. The criterium for rejecting the null hypothesis (absence of association or independence between variables) was fixed at $p \leq 0.05$.

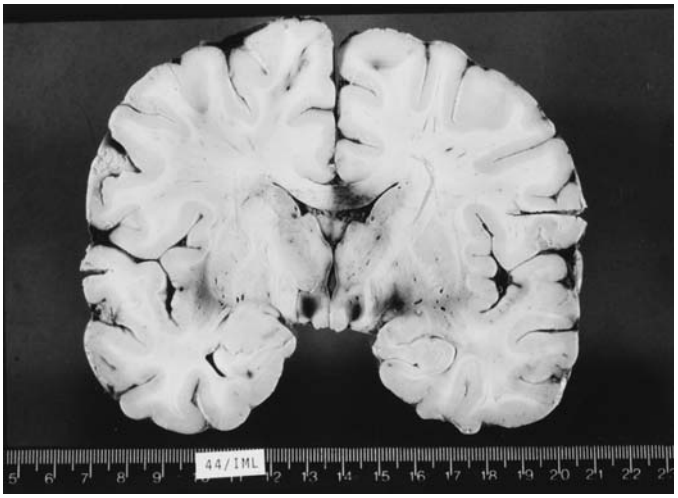

FIG. 1—*Diffuse axonal injury. Small hemorrhagic focus in and adjacent to the midline of the corpus callosum.*

FIG. 2—*Diffuse axonal injury. Small hemorrhagic lesion in the dorsolateral sector of the rostral midbrain* (left) *and pons* (right).

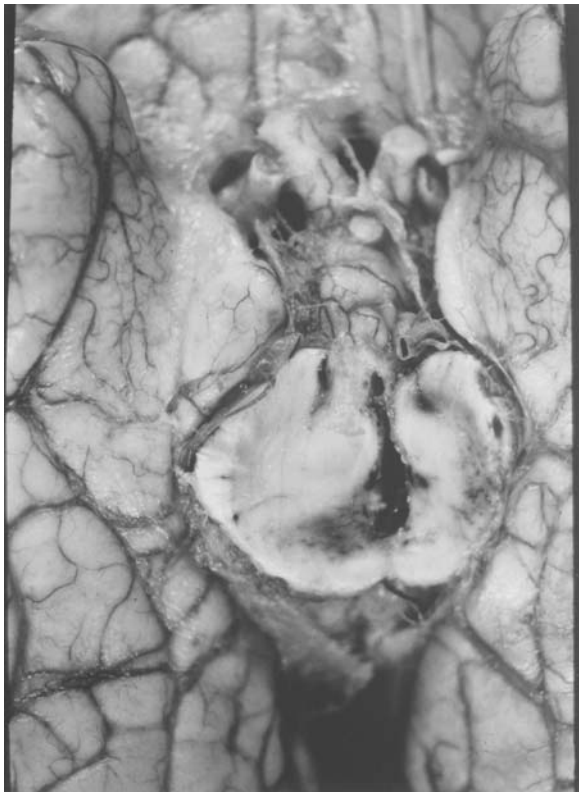

FIG. 3—*Duret's hemorrhage. A transtentorial hernia on the right has resulted in compression of the midbrain. The hernia is demarcated by a deep groove in the right parahippocampal gyrus. A large centrally located hemorrhage is present in the midbrain. The cerebral peduncle on the left is compressed and shows hemorrhagic necrosis.*

Results

The mean age of the 120 victims of fatal traffic accidents was 37.5 ± 18.3 years. The total number of male brains was 90 (75%) and of female brains, 30 (25.0%). Eighty-three patients (69.2%) died within the first 24 h, 41 (34.2%) at the site of the accident, and 42 (35%) after hospital admission; the remaining 37 (30.8%) sur-

vived between one and 28 days at the hospital. DAI was seen in 96 patients (80.0%); it was graded 1 in 21 (21.9%) patients, 2 in 49 (53.8%) patients, and 3 in 26 (27.1%) patients. Other head and extracranial lesions were represented by extradural hematoma in seven (5.8%) patients, acute subdural hematoma in 16 (13.3%), cerebral contusion in 67 (55.8%), burst lobes in 12 (10%), hypoxic brain damage in 23 (19.2%), morphological signs of increased intracranial pressure in 43 (35.8%), skull fracture in 63 (52.5%), fracture of limbs in 44 (36.7%), thoracic trauma in 45 (37.5%), abdominal trauma in 49 (40.8%), and pneumonia in 17 (14.2%) patients. In those patients that died at the site of the accident or very early after hospital admission, the cause of death was attributed to head trauma and/or internal hemorrhage, multiple fractures, and increased intracranial pressure. On the other hand, head trauma, increased intracranial pressure, hypoxic brain damage, and pneumonia, either isolated or in association, were the cause of death in the patients that survived between one and 28 days.

DVI was found in 14 of the 120 patients (11.7%). Of these, twelve (85.7%) were male and two (14.3%) female (Table 1). Their ages varied from 5 to 88 years. All had died within 24 h after the accident, eight patients (57.1%) at the site of the accident and six (42.9%) at the hospital. Five of the six patients that died after admission had GCS scores varying from 3 to 6, i.e., they were admitted in a state of coma, and in one patient the GCS score was 11. DAI was identified in all 14 patients with DVI. It was graded 2 in five patients (35.7%) and 3 in nine (64.3%). DVI was preferentially located in the rostral portion of the brain stem (midbrain and pons) and in the paramedian structures of the brain: fornix, basal ganglia, thalamus, and cerebellar white matter. In only one patient (Case 1) was DVI identified macroscopically in the form of multiple, punctiform hemorrhagic lesions in the cerebral white matter (Fig. 4). There was a significant statistical correlation between DVI and DAI (Table 2).

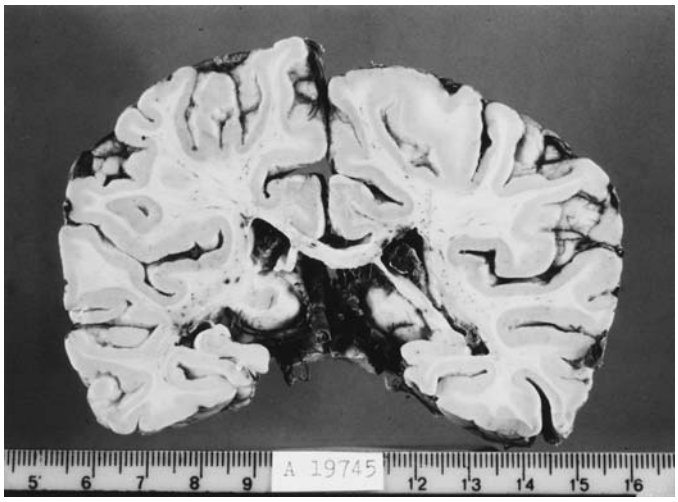

FIG. 4—*Diffuse vascular injury. Multiple, punctiform hemorrhagic lesions in the cerebral white matter.*

TABLE 2—*Correlation between diffuse vascular injury (DVI) and diffuse axonal injury (DAI) in 120 victims of fatal road traffic accidents.*

	DAI				
	Present		Absent		
DVI	*n*	%	*n*	%	Total
Present	14	14.6	0	0	14
Absent	82	85.4	24	100.0	106
Total	96	100.0	24	100.0	120

$p = 0.02$ (Fisher's exact test).

Discussion

The identification of axonal injury can be made by hematoxylin-eosin staining and silver impregnation or by immunohistochemical

TABLE 1—*Sex, age, state of consciousness as evaluated by the Glasgow Coma Scale (GCS), grading of diffuse axonal injury (DAI), and topography of the hemorrhagic lesions in 14 patients with diffuse vascular injury, not including focal hemorrhagic lesions characteristic of DAI Grades 2 and 3 (respectively, in the corpus callosum and in the dorsolateral quadrant of the rostral brain stem) or secondary to increased intracranial pressure (Duret's hemorrhage in the midline of the tegmentum of the midbrain and pons).*

Case	Sex	Age (years)	GCS	DAI	Location
1	M	88	11	2	CWM
2	F	5	4	2	F, CN, P, GP, A, T, Md, Pons
3	M	67	NE	2	CWM, Md, Pons, CeWM
4	M	35	6	2	CN, T, CeWM
5	F	42	3	3	F, Md, Pons, CeWM
6	M	15	NE	3	Parasaggital CC, AC, F, SI, CN, P, A, T, Pons
7	M	39	NE	3	Sb, F, CN, P, GP, T, Me, CeWM
8	M	27	NE	3	CWM, F, P, T, Me
9	M	25	NE	3	CWM, Md, Pons, CeWM
10	M	28	NE	3	CWM, F, H, T, CeWM
11	M	45	4	3	Parasaggital CC, F, IC, T
12	M	59	NE	3	F, CN, P, GP, T, H, Md, Pons, Me
13	M	67	NE	3	F, AC, CN, P, GP, T, H, Md, Pons, CeWM
14	M	28	3	2	Parasaggital CC, F, CN, P, T, Md

A = amygdala; AC = anterior commissure; CC = cerebral cortex; CWM = cerebral white matter; CeWM = cerebellar white matter; CN = caudate nucleus; F = fornix; GP = globus pallidus; H = hypothalamus; IC = internal capsule; Md = midbrain; Me = medulla; P = putamen; Sb = subiculum; SI = substantia innominata; T = thalamus

NE = not evaluated (death at the site of the accident).

techniques that use antibodies to the β-amyloid precursor protein (9–13), 68-, 170- and 200-kDa neurofilament proteins (7,14), and other axonally transported proteins. The immunohistochemical techniques are comparatively much more sensitive than the conventional silver impregnation to demonstrate axonal injury (7,12). In addition, neurofilament proteins and the β-amyloid precursor protein have been shown to be effective markers of axonal damage in the form of focal axonal swellings within the first 1 to 2 h after head injury (11,15). In a comparative study using antibodies to nine different antigens (including β-amyloid precursor protein and neurofilament protein), immunostaining for β-amyloid precursor protein produced the most sensitive and reliable staining of axonal injury (16), although the authors used an antibody targeted exclusively to the 68-kDa neurofilament subunit.

The pathological finding of small, multiple hemorrhagic brain lesions of traumatic origin was first drawn attention to by Cassaca (1). He reported five patients with multiple, punctiform hemorrhagic lesions disseminated in the brain parenchyma. In none of the cases were skull fractures or important cortical contusions observable. On microscopic examination, the punctiform hemorrhagic lesions were found to be located around the blood vessels in Virchow-Robin's perivascular space.

In a study of 151 fatal head trauma victims from a neurosurgery unit, Adams et al. (4) found two cases (1.3%) with small, multiple hemorrhagic lesions scattered in the brain. Since both patients were unconscious from the moment they sustained the trauma and remained in a coma until they died (less than 12 h later), it was suggested by these authors that this pathological entity represents a different type of primary, diffuse brain injury that occurs at the moment of the impact, and that it could be more frequent in the studies including patients that had died immediately or within a few hours after the head trauma.

The frequency observed in our study was much greater than that indicated by Adams et al. (4). In our series, as in the cases reported by Tomlinson (2), DVI was found to be practically restricted to road traffic accidents and the cause of rapid death (within less than 24 h). Thus, the high frequency in our study could be attributed to the fact that all 120 patients were victims of road traffic accidents and had a short period of survival, with 69.2% dying within 24 h after the accident. This high rate of immediate and very early deaths in the present series confirms the severity of head and extracranial injuries in the studied patients.

All patients with DVI had died within 24 h after the accident. Should the eight patients that died at the site of the accident be added to the five who were admitted in a state of coma, we may conclude that 13 of the 14 patients showed severe, immediate alteration of consciousness. Case 1 was the only patient who was not admitted in a state of coma (GCS 11). This less severely altered consciousness on admission could be due to the fact that the hemorrhagic lesions in this patient were located only in the cerebral white matter, without involvement of the brain stem by any primary injury or any damage secondary to increased intracranial pressure. According to Graham and Gennarelli (17), multiple hemorrhages in the cerebral hemispheres, but not in the brain stem, are compatible with a survival of up to several hours. Our Patient 1 confirms that there may be a spectrum of fatal vascular damage, even at the severe end of the scale. The frequent association between DVI and DAI is also in accord with the short period of survival and low conscious level on hospital admission. Such results confirm the hypothesis advanced by Adams (18,19) that DVI is a primary, diffuse brain lesion, i.e., it occurs at the moment the trauma is sustained.

Although DVI can often be identified macroscopically, many more microhemorrhages are apparent on microscopic examination (4). It occurs predominantly in the white matter of the frontal and temporal poles, in and adjacent to the thalamus and in the brain stem (18,19). In this latter location, it is mainly found in the subependymal tissue surrounding the cerebral aqueduct and the floor of the rostral portion of the fourth ventricle (2,3). In our series, only in one case was DVI identifiable macroscopically, which demonstrates the importance of microscopic examination of the brains of victims of fatal head trauma.

Experimental studies have shown that there is a direct response of the cerebral microvasculature to lateral head acceleration. A number of microvascular changes, such as the extravasation of blood in a small number of blood vessels in all parts of the brain, increased endothelial pit/vesicle activity, development of endothelial microvilli, and swelling of perivascular astrocytes have been reported after lateral head acceleration in the non-human primate (20,21). Therefore, similar to DAI, DVI appears to depend on long-duration, high-level acceleration and is practically restricted to road traffic accidents (19,22). This would explain its association with severe (Grades 2 and 3) DAI. Only acceleration of sufficient intensity to cause rupture of the blood vessels in the corpus callosum and dorsolateral brain stem could also lead to vascular injury in other regions of the brain. Therefore, DAI and DVI would depend on the same mechanism, with the degree of axonal and vascular disruption being determined by the intensity of the accceleration rupture (5). DAI of Grades 2 and 3 can be defined as a macro- or microhemorrhage, located in the corpus callosum, usually in the paramedian position, and in the dorsolateral quadrant of the midbrain and pons, respectively (6,19,22). DVI would be the association of this hemorrhage in the corpus callosum and/or rostral brainstem with widespread, multiple, small or microhemorrhages in the cerebral white matter and/or in cerebellar white matter, cerebral cortex, basal ganglia, thalamus, and brain stem. Thus, our results show a relationship between DVI and DAI that suggest there may be a spectrum or at least a continuum between these entities as distinct from DVI being a separate entity.

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