PARTICULAR MORPHOPATHOLOGICAL ASPECTS OF THE NEW-BORN'S CEREBRAL HEMORRHAGE

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Abstract

Introduction: Cerebral hemorrhage represents the main cause of death of the premature new-born and a rare one for the new-born in term.

Aim: This study pinpoint that the neuronal damages are present long time before the labour, to a important cohort of neonatal death, especially premature.

Material and method: Study group includes 153 new-borns deceased to our Children Hospital, during 2002-2007, wich main cause of death was the cerebral hemorrhage.To all studied cases we performed a complet morphopathological examination of the brain.We proceeded to a inventory of the brain injuries, for every cases

Results: The analysis of the casuistry guided us to elaborate the following observations:

• a meaningful ratio of neonatal mortalityt hrough cerebral hemorrhage, is 31.35%;
• an meaningful incidence of the fulminating form with decease in the first 7 days of life;
• a diffuse localization of the injuries to premature borns, proportionate with the degree of the immaturity;
• a constant harm of the undercortical structures, in the periventriculer, basilar, and cerebellar zones;
• the coexistence of some old injuries(ante-natal) with others recent (post-natal) is a frequente situation to the dominant group, with small weight at birth,(63.4%);
• a lesional association to the 60% of the cases, involving, in the next order : hemorrhage of the cerebral artery, ventricular inundation, vascular thrombosis

Conclusions:

1. Cerebral hemorrhage represents the main reason of the decease of the premature new-born;
2. The impact of the ante-natal time on the immature fetal brain generates a stronglesional potential;
3. The multiple sites in the territory of the cerebral artery is prevalent in the morphopatho-logical aspects of the cerebral hemorrhage.

Key words: cerebral hemorrhage, new-borns

Introduction

Newborn's cerebral hemorrhage occurs in hypoxic-ischemic complex brain injuries and not as an obstetric mismanagement of term birth anoxia[2,3,6]. We consider that most cases of neonatal hypoxic-ischemic encephalopathy have antenatal insults, difficult to pinpoint[2,8,9,]. We already can be sure about a great number of vasculopathy alteration, such as :

- changes in the blood flow direction in circle of Willis[8];
- decreased blood-flow velocity in the veins;
- changes in the flow of the right vertebral artery, with isolated hemorrhagic lesions;
- neonatal thrombotic vessels;
- vessels occlusion by an embolus with obstruction of the middle cerebral artery.

Aim

This study pinpoint that the neuronal damages are present long time before the labour, to a important cohort of neonatal death, especially premature.

Material and methods

Study group includes 153 new-borns deceased to our Children Hospital , during 2002-2007, wich main cause of death was the cerebral hemorrhage.To all studied cases we performed a complet morphopathological examination of the brain.We proceeded to a inventory of the brain injuries, for every cases.

Specimens Samples were obtained from the brains in the time of autopsy, from all 153 newborns who died with cerebral hemorrhage.After fixation in 4% buffered formalin, for 24-48 hours, we procedeed sections (4 μm thick), and embedded in paraffin.We used routinely technique Hematoxylin–Eosin(HE), for microscopic analyses.

Immunohistochemistry Samples sections were rehydrated, washed and then rinsed in PBS (pH 7.2).Sections were incubated with 3% hydrogen peroxide solution for 5 minutes, then washed with PBS. After endogenous peroxidase inhibition and antigen retrieval, the sections were incubated with the primary antibodies.

Formalin-fixed, paraffin-embedded tissues were incubated so the slides could react with a labelled avidin-biotin complex, peroxidase-labelling detection system(Vector Universal Elite kit) and then treated with 3,3'-diaminobenzidine-peroxidase substrate solution, as chromogen (DAB Tablets, S3000-Dakopatts, Glostrup Denmark) until color was visualized.It was done using the method EmVision Dual Link-HRP.All reagents and supplied for the technique were from Dako, Denmark.

The primary antibodyies, which were: the monoclonal rabbit anti-GAFP (Dakopatts, Glostrup Denmark) mouse anti-S-100 (Dako, polyclonal, code

N1573, ready-to-use), anti-CD68 (Dako, PG-M1) and the monoclonal mouse antivimentin (Dako, clone V9).The negative control reagent used for LSAB2 was Universal Negative Control, Rabbit (code N1699).

Sections were washed twice in distilled water to stop the reaction, then counterstained in hematoxylin, washed, dehydrated, cleared in xylene, mounted with DPX, and glass cover-slipped.

Sections were examined under oil immersion with a ×100 objective on a Nikon Eclipse E–400 microscope, and images were captured using a Coolpix 995 digital camera and a DN–100 digital imaging system. Histological sections were reviewed independently by two pathologists, and then discussed for consensus.

Results

The analysis of the casuistry guided us to elaborate the following observations:

- a meaningful ratio of neonatal mortality through cerebral hemorrhage, is 31.35%(Table 1);
- a meaningful incidence of the fulminating form with decease in the first 7 days of life (Table 1);
- a diffuse localization of the injuries to premature borns, proportionate with the degree of the immaturity (Table 2);
- a constant harm of the undercortical structures, in the periventricular, basilar, and cerebellar zones (Fig.1, 2 and Table 3);
- the coexistence of some old injuries (ante-natal) with others recent (post-natal) is a frequente situation to the dominant group, with small weight at birth,(63.4%) (Fig. 3,8 and Table 3) ;
- a lesional association to the 60% of the cases, involving, in the next order : hemorrhage of the cerebral artery , ventricular inundation, vascular thrombosis (Table 3);
- immunohistolabeling for the brain and the choroid plexus, revealed intens positivity for:

  - **G.F.A.P. -** glial fibrillary acidic protein – that is thought to help to maintain astrocyte mechanical strength; it is also involved in many cellular functioning processes, such as cell structure and movement, cell communication, and the functioning of the blood brain barrier (Table 2, Fig. 5, 9);
  - **CD 68** has also intes positivity, especially in the brain lesions (Table 2, Fig. 4, 10); CD68 is a glycoprotein which binds to low density lipoprotein, predominantly a late endosomal protein but is expressed also on the cell surface . It is predominately expressed in cytoplasmic granules of monocytes/macrophages, dendritic cells, and granulocytes.
  - **Protein S100** – we saw intens positivity in the brain, (Table 2, Fig.6); protein S100 is secreted by cultured astroglial cells and functions as a trophic factor for a number of neuronal cell types, stimulating the differentiation of immature neurons. It promotes the survival of these cells in vitro and induces the outgrowth of neurites.
  - **Vimentin –** shows negativity, for the brain and choroid plexus, (Table 2, Fig. 7, 12). Vimentin plays a significant role in supporting and anchoring the position of the organelles in the cytosol. Vimentin is attached to the nucleus, endoplasmic reticulum, and mitochondria, either laterally or terminally.In general, it is accepted that vimentin is the cytoskeletal component responsible for maintaining cell integrity.

Table 1 – Prematures and age at death (in days) of the newborns with cerebral hemorrhage.

STUDY YEARS	PREMATURES (from all newborns)	AGE AT DEATH (days)			
		1 -7	8 -14	15 - 21	22 - 30
2002	16	19	4	1	1
2003	22	21	5	3	1
2005	19	14	4	5	
2006	15	16	4	2	1
2007	12	12	3	3	3
TOTAL	105	106	28	14	5

Table 2 - Particular macroscopical aspects of neonatal cerebro-vascular injuries are often associated and variable.

MARKER STUDY	CASES / YEAR	BRAIN						CHOROID PLEXUS & EPENDIMA					
		ASTROCITE		MICROGLIA		NEURAL DEATH		ASTROCITE		MICROGLIA		NEURAL DEATH	
		YES	NO	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO
H&E	2/2002	+		+		+		+		+		+	
GFAP	4/2003	+		+			+	+		+			+
prot. S100	4/2005	+			+		+	+			+	+	
CD68	10/2006	+		+		+		+		+		+	
Vimentine	11/2007		+		+		+		+		+		+
TOTAL	31	4	1	3	2	2	3	4	1	3	2	3	2

Table 3 - Immunohistolabeling for brain, choroid plexus and ependima.

STUDY YEARS	Cortical infarcts and petechial white-matter hemorrhage	Multifocal hemorrhagic white-matter infarcts	Choroid plexus and subependymal thrombosis	Germinal matrix and intraventricular hemorrhage
2002	14	5	15	12
2003	17	8	18	15
2005	16	8	14	9
2006	15	3	16	11
2007	10	3	10	6
TOTAL	91	38	86	78

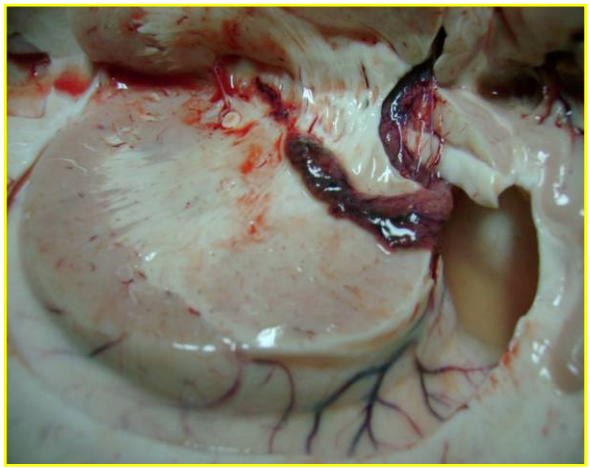

Fig. 1 Brain – Thrombotic choroid plexus with vessels thrombosis subependimal.

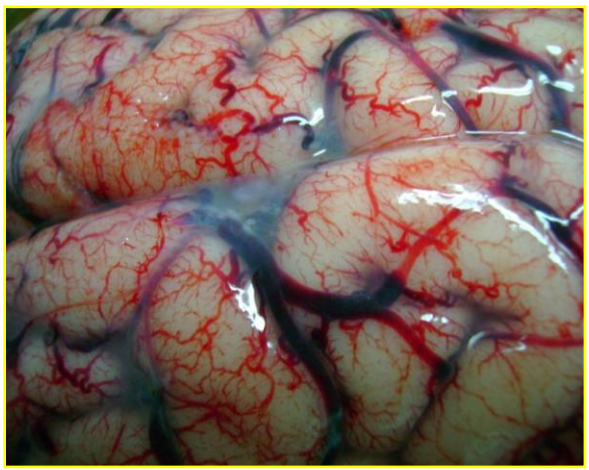

Fig. 2 Brain - Thrombotic generalized.

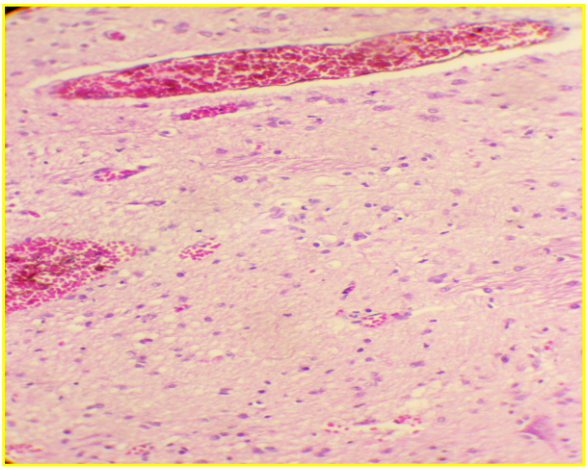

Fig. 3 Brain - H.E.

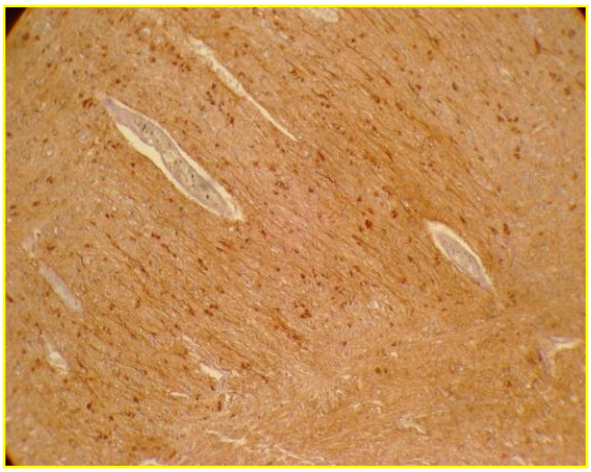

Fig. 4 Brain - CD 68.

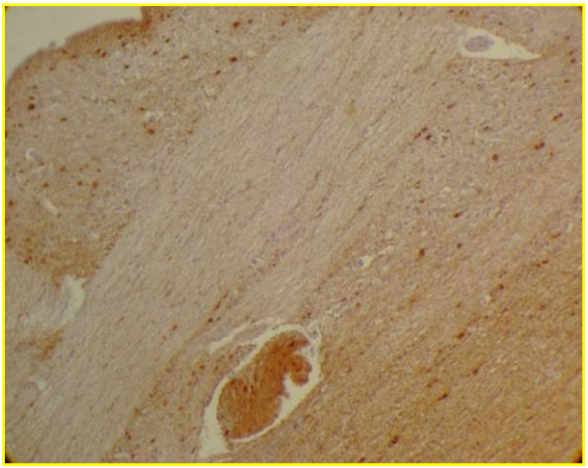

Fig. 5 Brain - G.F.A.P.

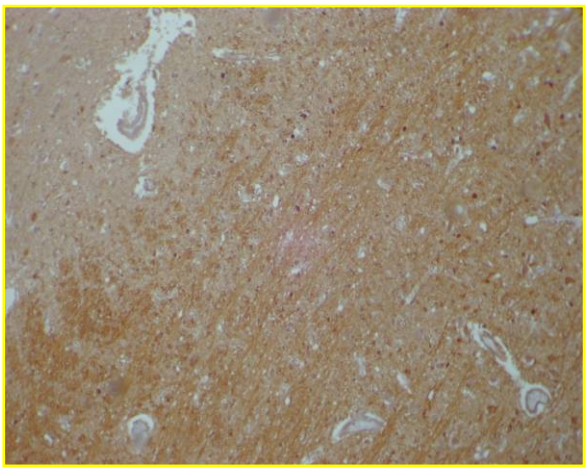

Fig. 6 Brain - Protein S 100.

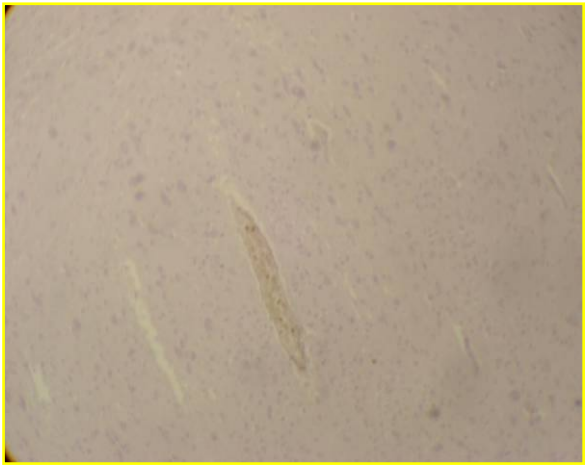

Fig. 7 Brain – Vimentin.

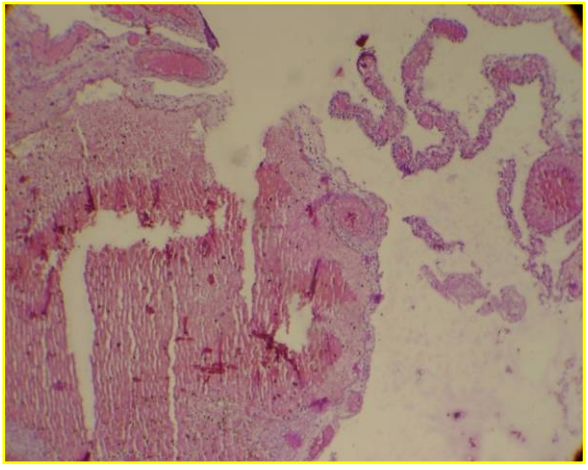

Fig. 8 Choroid plexus - H.E.

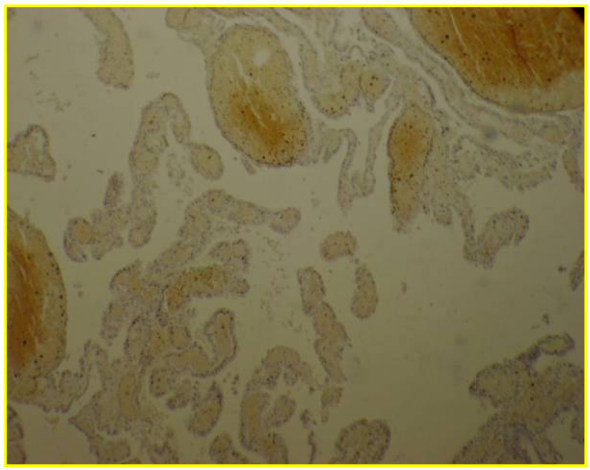

Fig. 9 Choroid plexus - G.F.A.P.

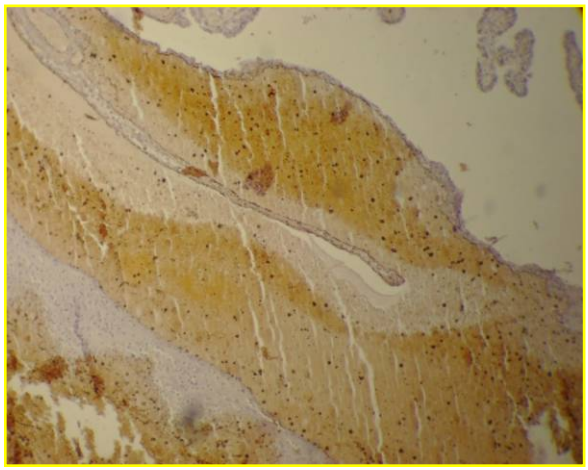

Fig. 10 Choroid plexus - CD 68.

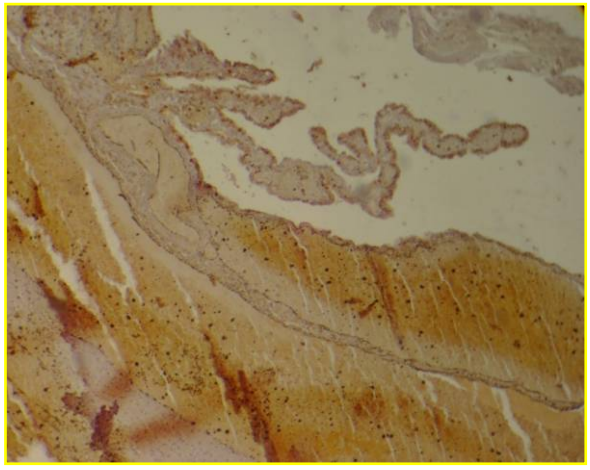

Fig. 11 Choroid plexus - Protein S 100.

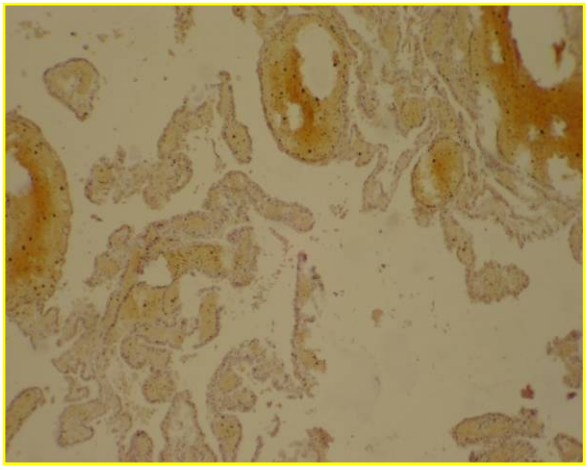

Fig. 12 Choroid plexus – Vimentin.

Discussion

Very few studies are correlating the pathological findings in neonatal brains with detailed pathological clinical systematical based[7].

Usually related with the reactions to signs of birth asphyxia, in the present study we try to explane which are the neuronal and axonal injury in these infants, and find the basis for neurological deficits[2]. We intend also to investigate these brains for specifical markers of neuronal injuries in neonates(precursors of protein detected in future by noninvasive methods).Usually located in the cerebral white matter and internal capsule, positivity were significantly correlated with the features of birth asphyxia, particularly a history of neonatal hemorrhage.Immunocytochemistry for GFAP is not difficult to be labeled, systematically, because it is very important to help us to select the presence together, of recent and older damages, particularly in preterm infants[9,10].

Conlusions

1. Cerebral hemorrhage represents the main reason of the decease of the premature new-born[4];
2. The impact of the ante-natal time on the immature fetal brain generates a strong lesional potential[5.11,12];
3. The multiple sites in the territory of the cerebral artery is prevalent in the morphopathological aspects of the cerebral hemorrhage[10].
4. In the absence of trauma, the mechanism of hypoxia/ischaemia remains the main cause[1,8].

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