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Original Article

New Autopsy Findings in Different Brain Regions of a Preterm Neonate With Kernicterus: Neurovascular Alterations and Up-regulation of Efflux Transporters

Maria A. Brito PhD [a,b,*], Pedro Pereira MSc [c,d], Cândida Barroso MD [c,d], Eleonora Aronica MD, PhD [e], Dora Brites PhD [a,b]

[a] *Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal*
[b] *Department of Biochemistry and Human Biology, Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal*
[c] *Laboratory of Neuropathology, Centro Hospitalar Lisbon Norte, Hospital de Santa Maria, Lisbon, Portugal*
[d] *Neuromuscular Unit, Institute of Molecular Medicine, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal*
[e] *Department of (Neuro)Pathology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands*

ABSTRACT

BACKGROUND: Kernicterus is an irreversible brain damage caused by bilirubin deposition in selective brain regions. Sick and preterm infants with hyperbilirubinemia are particularly susceptible to the condition. **METHODS:** We studied autopsied brain tissue from a premature female infant with kernicterus with a bilirubin:albumin molar ratio of 1.0, hypoxia, acidosis, and seizures. The patient, previously described as having cerebellar axon/myelin loss and angiogenic sprouting, was assessed for histopathological features in brain regions less investigated, such as hippocampus and corpus striatum. Results were compared with age-matched controls. **RESULTS:** Increased blood vessel density with poorly defined lumen structures was observed in the mesencephalon, pons, and medulla oblongata, and, more predominantly, in the corpus striatum and hippocampus. These two regions exhibited increased expression of vascular endothelial growth factor, paralleled by vascular endothelial growth factor receptor-2, and albumin extravasation into the brain parenchyma. No similar findings were observed in the nonjaundiced babies with hypoxia that served as controls (one preterm with sepsis and a term infant with pneumonia). We found increased cellular expression of multidrug resistance–associated protein 1 and P-glycoprotein in the hippocampus, known as defensive mechanisms against bilirubin-induced cytotoxicity. Increased density of blood vessels and microvascular permeability, together with parenchymal albumin, may have contributed to increasing the brain content and retention of bilirubin, a condition implicated in kernicterus disease. **CONCLUSIONS:** This novel finding in a premature baby with kernicterus and associated risk factors deserves to be investigated in similar patients to better understand the less-well described effects of bilirubin-induced neurological sequelae in preterm infants.

Keywords: albumin, blood-brain barrier, corpus striatum, efflux transporters, hippocampus, kernicterus, microvascularization, vascular endothelial growth factor

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Introduction

Neonatal jaundice is a common condition, affecting 60% to 85% of newborn infants.[1] In some circumstances, increased bilirubin levels in the circulation may cause bilirubin-induced neurological dysfunction and chronic bilirubin encephalopathy, known as kernicterus. The term refers to the yellow staining of the basal ganglia by unconjugated bilirubin (UCB), only recognized at autopsy. Although decreasing in prevalence in developed countries, the condition continues to be reported throughout the world.[2,3] The clinical expression of kernicterus relates to its selective damage of the globus pallidus, substantia nigra reticulata, subthalamic nucleus, brainstem auditory, vestibular and oculomotor nuclei, hippocampus, and

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* Communications should be addressed to: Dr. Brito; Faculdade de Farmácia da Universidade de Lisboa; Av. Prof. Gama Pinto; 1649-003 Lisboa, Portugal.
E-mail address: abrito@ff.ul.pt

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cerebellum,[4] together with brain edema.[5] Histological findings include extensive apoptotic changes and necrosis of neurons in the affected brain regions and multiple minute calcifications in the cerebral white matter.[6] In a recent case report of a preterm infant with acute kernicterus with signs of hypoxia and suspected sepsis, features such as axon and myelin loss, angiogenic sprouting, and neuronal increase of vascular endothelial growth factor (VEGF) and its receptor (VEGFR-2) were observed.[7] On the other hand, altered expression of neurotransmitters, neuropeptides, and calcium-binding proteins in the basal ganglia and the substantia nigra has been reported in some cases of bilirubin encephalopathy.[8] With the development of magnetic resonance imaging techniques, the brain lesions characteristic of kernicterus started to be identified.[9] However, further detailed information on the histological and functional disturbances in kernicterus cases may add to our understanding of kernicterus neuropathology. Indeed, almost no histopathological data have been reported for other brain regions, such as the hippocampus and corpus striatum, also injured by UCB, and there is scarce information concerning the expression of the membrane efflux proteins P-glycoprotein (Pgp; adenosine 5'-triphosphate-binding cassette b1) and multidrug resistance–associated protein (MRP1or Mrp1 in rodents; adenosine 5'-triphosphate-binding cassette c1). Only one article discussed hippocampal sclerosis in kernicterus by magnetic resonance imaging,[10] whereas another reported the up-regulation of Pgp in brain microvessels and the down-regulation of Mrp1 in choroid plexi isolated from jaundiced jj Gunn rat pups used as an experimental model of kernicterus.[11] Moreover, it has also been suggested that, if blood-brain barrier disruption occurs, UCB bound to albumin may penetrate the cerebral extracellular space, further contributing to bilirubin-induced neurological dysfunction and kernicterus.[12,9] However, no reports have focused on the hypothetical presence of albumin in the kernicteric human brain.

The absence of this information in previous neuropathological reports, including our previous publication on the alterations in cerebellum myelin pattern in a preterm infant diagnosed with kernicterus,[7] led us to investigate these features in other autopsy materials collected from the same patient. Therefore, we assessed histological and immunoreactive alterations related to microvascularization and albumin extravasation into the parenchyma, as well as to the expression of VEGF, VEGFR-2, Pgp, and MRP1 proteins in the corpus striatum, hippocampus, mesencephalon, pons, and medulla oblongata.

Material and Methods

Case summary

A preterm (32 5/7 weeks) girl was born weighing 1600 g with Apgar scores of 6 at 1 minute and 7 at 5 minutes, requiring artificial ventilation. Focal pulmonary infiltrates were detected on chest x-ray, and antibiotic therapy was initiated on the first day of life for suspected sepsis. Total serum bilirubin was 13.1, 28.8, and 21.4 mg/dL on the first, second, and fourth postnatal days, achieving an albumin/bilirubin molar ratio of 1.0 in the second day of life; intensive phototherapy was initiated on the second day of life. Her clinical condition rapidly deteriorated, with high-pitched cry, poor suckling, hypotonia, hypotension, bradycardia, metabolic acidosis, and the development of generalized seizures despite pharmacological treatment initiated on the third postnatal day. Following progressive cardiorespiratory problems, the child died on the fourth day of life. She had no ABO/Rh isoimmunization and cultures were negative, although performed after administration of antibiotics. Autopsy revealed signs of meconium aspiration. No evidence of infection or hemolysis was found in any of the other organ systems and the clotting parameters were normal. Her cerebral cortex displayed normal gyri and sulci. Coronal sections of the cerebral hemispheres revealed marked yellow staining of hippocampus and thalamus (Fig 1A), together with globus pallidus and subthalamic nuclei, vestibular nuclei, cochlear nuclei, and medial lemnisci of the medulla oblongata (Fig 1B). The yellow staining was observed at autopsy on fresh sections (i.e., hippocampus), but also after fixation;

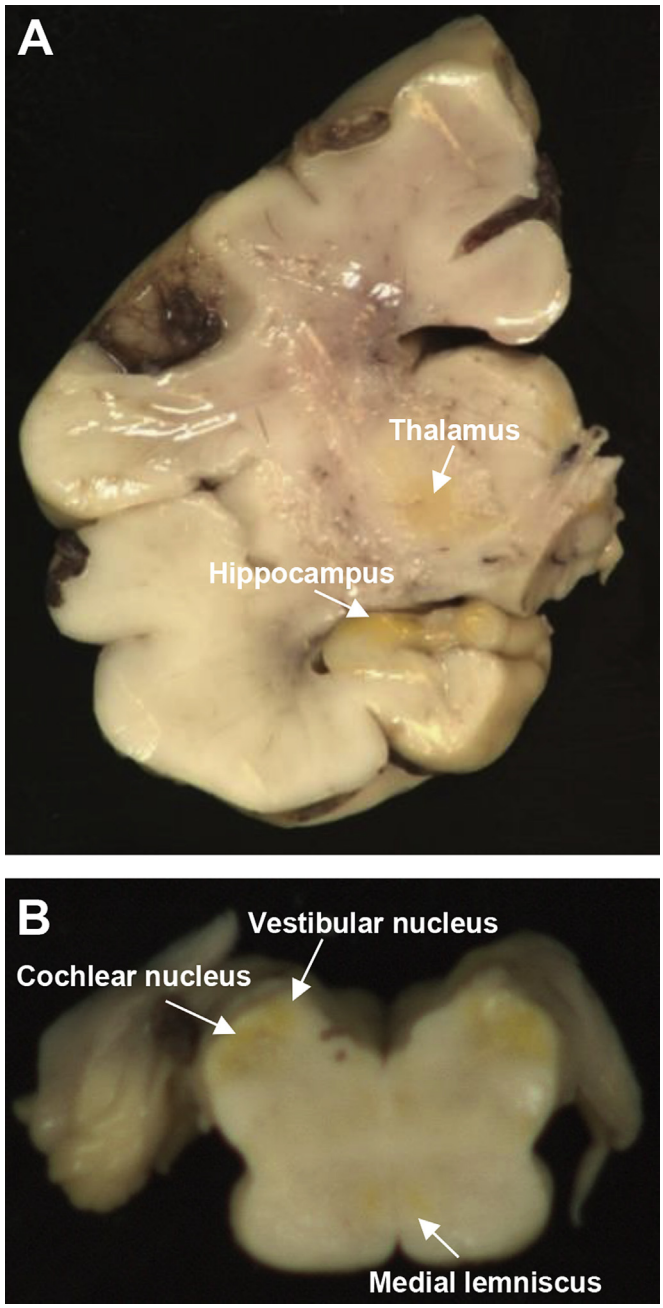

FIGURE 1.
Macroscopic examination of the jaundiced preterm infant brain at autopsy reveals the characteristic regional pattern of kernicterus yellow staining. (A) Coronal section of the cerebrum. (B) Transverse section of the medulla oblongata.