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ORIGINAL ARTICLE

Proinflammatory Cytokines, Enolase and S-100 as Early Biochemical Indicators of Hypoxic-Ischemic Encephalopathy Following Perinatal Asphyxia in Newborns

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Key Words
enolase;
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Background: Estimation of the neurological prognosis of infants suffering from perinatal asphyxia and signs of hypoxic-ischemic encephalopathy is of great clinical importance; however, it remains difficult to satisfactorily assess these signs with current standard medical practices. Prognoses are typically based on data obtained from clinical examinations and

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neurological tests, such as electroencephalography (EEG) and neuroimaging, but their sensitivities and specificities are far from optimal, and they do not always reliably predict future neurological sequelae. In an attempt to improve prognostic estimates, neurological research envisaged various biochemical markers detectable in the umbilical cord blood of newborns (NB). Few studies examining these biochemical factors in the whole blood of newborns exist.

Thus, the aim of this study was to determine the expression and concentrations of proinflammatory cytokines (TNF-α, IL-1β and IL-6) and specific CNS enzymes (S-100 and enolase) in infants with perinatal asphyxia. These data were compared between the affected infants and controls and were related to the degree of HIE to determine their utilities as biochemical markers for early diagnosis and prognosis.

Methods: The levels of the proinflammatory cytokines and enzymes were measured by enzyme-linked immunosorbent assay (ELISA) and Reverse Transcription polymerase chain reaction (RT-PCR).

Results: The expression and serum levels of the proinflammatory cytokines, enolase and S-100 were significantly increased in the children with asphyxia compared with the controls.

Conclusion: The role of cytokines after hypoxic-ischemic insult has been determined in studies of transgenic mice that support the use of these molecules as candidate biomarkers. Similarly, S-100 and enolase are considered promising candidates because these markers have been correlated with tissue damage in different experimental models.

1. Introduction

Perinatal hypoxic-ischemic encephalopathy (HIE) is a major cause of perinatal mortality and the development of neurological sequelae of varying severity, such as cerebral palsy, seizures, visual impairment, mental retardation, learning impairment, and epilepsy.[1]

Lai and Yang[2] showed that 15—25% of affected newborns die during the postnatal period and that 25% develop severe and permanent neuropsychological sequelae.

The incidence of neurological sequelae in survivors is 20-45%; however, 40-60% of cases are severe. The Sarnat grading scale is used to classify cases of HIE. Newborns with mild HIE (Grade I) usually have a normal outcome. Approximately 80% of patients with Grade II encephalopathy recover; however, the mortality rate is 3%, and 20—45% experience neurological sequelae. Patients with severe HIE (Grade III) have a 50% mortality rate, and survivors have severe neurological consequences.[3]

Several interrelated pathophysiological mechanisms involving excitotoxicity, intracellular calcium, free radicals, nitric oxide (NO), cytokines, and apoptosis may explain HIE.[4] Microglia are the resident immune cells in the brain, and microglial activation is the initial step in inflammatory responses of the central nervous system (CNS) to various stimuli, including stroke.[5,6] Moreover, microglial cells induced by HIE produce proinflammatory cytokines that cause damage to the overall structure of the CNS.[7] Asphyxiated infants have high concentrations of interleukin-6 (IL-6) and IL-8 in their cerebrospinal fluid (CSF), and the extent of brain damage is directly related to the concentrations of these cytokines.[8] Other biochemical markers, such as neuron-specific enolase (NSE) and S-100B, can also be present at increased levels in blood, especially in disorders with acute brain damage such as traumatic brain injury,[9] cardiopulmonary bypass surgery,[10] and cardiac arrest.[11]

Various biochemical markers that are detectable in blood and/or CSF have been investigated for improvement of neurological prognostics. Biomarkers may help to determine when injuries occur; this is important because hypoxic-ischemic injury often begins *in utero*, and if too much time has elapsed since the initial brain injury, the neonate will not benefit from treatment. This may explain why some neonates with HIE do not respond to treatment.

For a biomarker to be useful, it should be easily isolated from CSF, blood, or urine and identify infants with brain injury during the first few hours of life. Several biomarkers have been examined as outcome indicators, but none are routinely used in the clinic.

Therefore, this study aimed to evaluate the possible molecules involved in neuronal damage subsequent to hypoxic-ischemic injury by analyzing the concentrations and expression of the inflammatory cytokines tumor necrosis factor alpha (TNF-α), IL-1β, and IL-6 in HIE patients. Additionally, we measured the serum levels of enolase and the neurotropic factor S100 protein.

2. Methods

2.1. Control group

A total of 32 term newborn infants with a median gestational age of 40 weeks (range: 37—42 weeks) were selected from the Gynecology and Obstetrics Hospital (Mexican Institute of Social Security) before the start of the study. All control infants had an Apgar score of $\geq$ 9 at 1, 5, and 10 minutes and were born between 2010 and 2012 after an uncomplicated vaginal delivery, with a mean umbilical

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