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Neuroinflammation-Related Encephalopathy in a Preterm Infant Following Exposure to Maternal Diabetic Ketoacidosis

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A pregnant woman with new onset type 1 diabetes and ketoacidosis delivered an infant at 28 weeks of gestation who died with multiple organ failure and severe cerebral vasculopathy with extensive hemorrhage, diffuse microgliosis, and edema. This illustrates that antenatal metabolic and inflammatory stressors may be associated with neonatal encephalopathy and cerebral hemorrhage.

In 1861, William J. Little implicated events during the birth process as potential etiologies of cerebral palsy, whereas in 1897, Sigmund Freud suggested that prenatal factors may be responsible. This debate remained unresolved until 1984 when the Collaborative Perinatal Project provided evidence that only a small proportion of cerebral palsy stemmed from events arising during labor and delivery¹. Several smaller subsequent studies supported this conclusion^{2–5}. Furthermore, the failure of cerebral palsy incidence rates to decline despite advances in perinatal medicine⁶ supports the concept that underlying disorders occurring prior to birth mediate the majority of abnormal neurodevelopmental outcomes in children^{2–5}, in agreement with Freud's hypothesis.

Intrauterine pathophysiological factors that may contribute to cerebral palsy include hypoxic-ischemic injury, inflammation, and immunological and coagulation abnormalities^{7–15}. Inflammation is now recognized as an important contributor to pregnancy related complications^{13, 16} and is at the core of both Type 1 and Type 2 diabetes. Type 1 diabetes is an autoimmune disorder^{17, 18} whereas Type 2 diabetes is an insulin resistance disease. Type 1 or Type 2 diabetes during pregnancy exposes the fetus to a hyperglycemic inflammatory milieu.

In this report, we present an infant born to a mother who developed new onset Type 1 diabetes during pregnancy and presented with severe diabetic ketoacidosis in the perinatal

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period. The infant died shortly after birth from extensive brain injury and multi-organ failure. This case illustrates that neonatal brain injury can result from prenatal maternal metabolic, inflammatory and immune pathophysiological factors.

Report of a Case

The mother was a 19-year-old G3, P0111 woman who presented at 28 weeks and 4 days of gestation with flu-like symptoms, including a sore throat, body aches, nausea, vomiting, and abdominal pain, but without chills or diarrhea. The pregnancy was associated with recurrent urinary tract infections. However, routine prenatal blood and urine studies were normal and fetal movements were positive. A Level II prenatal ultrasound was interpreted as showing multiple choroid plexus cysts, but subsequent ultrasound examinations were normal.

Maternal admission physical examination was notable for tachypnea, tachycardia, and a body mass index of 31.6 kg/cm.² The arterial pH was 7.21 ; pCO₂: 15 mmHg; base excess: -18.9 mEq/L; serum sodium: 139 mEq/L; serum glucose: 335 mg/dL; hemoglobin A1C: 8.3% and C-peptide: 3.8 ng/ml. The metabolic panel revealed an anion gap with acidosis and 2+ urinary ketones. The patient was diagnosed with diabetic ketoacidosis secondary to adult onset type 1 diabetes mellitus. The fetal heart rate was < 60 beats/minute, prompting an emergency cesarean section and deferral of further maternal medical work-up.

The female infant had an initial Apgar score of 0. The infant was intubated and positive pressure ventilation and chest compressions were initiated 2 min 44 seconds after birth due to inadequate response to prior resuscitative efforts. After intubation, the first epinephrine dose was administered via the endotracheal tube, and two subsequent intravenous doses were given via an umbilical venous catheter. After 16 minutes of resuscitation, heart rate improved, chest compressions were discontinued, and the infant was admitted to the neonatal intensive care unit (NICU) on an FiO₂ of 0.90. Arterial cord pH was 6.67, venous pH was 6.91, and the base excess was -22.7 mEq/L

The initial examination in the NICU revealed a normal appearing premature female infant with a birth weight of 1450 grams (90th percentile). The infant had profound, diffuse hypotonia, no spontaneous activity, myoclonus, and fixed dilated pupils. Clinical interventions included synchronized intermittent mandatory ventilation (FiO₂ 0.40–0.90) for respiratory depression, administration of two doses of surfactant and one dose of caffeine, and dopamine infusion for hypotension. Initial laboratory studies revealed a pH of 6.94, PO₂ of 49.5 mmHg, PCO₂ of 128.7 mmHg, base excess of -20 mEq/L, serum glucose of 229 mg/dL, serum bicarbonate of 12 mEq/L, sodium of 139 mEq/L, creatinine of 1.36 mg/dL, and a normal complete blood count. Ketones, lactate and insulin levels were not measured. At 3 hours, a postnatal cranial ultrasound revealed normal midline structures and cerebral ventricles, cerebral echogenicity and no evidence of intraventricular hemorrhage.

Despite aggressive support, the infant remained ventilator-dependent with persistent metabolic acidosis, high FiO₂ requirements, bradycardia and anuria. Laboratory findings included serum creatinine (1.71 mg/dL), aspartate aminotransferase (410 U/L), alanine aminotransferase (59 U/L), and alkaline phosphatase (599 U/L) levels. A second cranial

ultrasound examination performed at 18 hours demonstrated diffusely heterogeneous echogenicity throughout the cerebrum, most likely reflecting severe ischemic injury. In light of the increased demands for respiratory support, multi-organ failure, and high likelihood of a poor neurological outcome, consent was obtained from the family to re-direct care. The infant expired at 24 hours of age, shortly after she was extubated. Consent was obtained to perform a full autopsy.

General Autopsy Examination

The post mortem examination revealed a normally developed female infant weighing 1475 g (> 90th percentile). Evidence of multiple organ failure was manifested by cardiomegaly with left ventricular hypertrophy, hepatomegaly with massive congestion, diffuse bilateral renal intramural hemorrhages, and serous body cavity effusions. Thymic involution with petechial hemorrhages reflected responses to stress. The pancreas had normal acinar development, islet distribution, and islet architecture, and showed no evidence of fibrosis or inflammation, including insulinitis and peri-insulinitis. In addition, immunohistochemical staining for insulin confirmed the absence of islet cell hyperplasia.

The placenta weighed 327 gm (75th-90th percentile), had characteristic preterm histological features with superimposed pathologies reflecting maternal vascular malperfusion, manifested by hypertrophic type decidual vasculopathy with focal fibrinoid necrosis of vessel walls, accelerated villous maturation, basal plate multinucleated trophoblast, and multifocal villous edema.

Postmortem Neuropathological Examination

The brain weighed 196 grams and its development was appropriate for gestational age. The brain exhibited diffuse edema with early encephalomalacia, extensive symmetrical hemorrhage with early cavitation throughout cerebral white matter and subcortical nuclei, leaving a thin rind of cortical parenchyma intact. Less severe hemorrhage symmetrically involved all levels of brainstem. Of note is that the germinal matrix zone, ventricular cavities, choroid plexus, and cerebellum were spared (Figure 1).

Formalin-fixed paraffin-embedded sections of the hippocampus, neostriatum including germinal matrix, frontal, parietal, and occipital cortex and white matter, periventricular white matter, choroid plexus, brainstem (all levels), cerebellum, and spinal cord stained with Luxol fast blue, hematoxylin and eosin revealed three main pathological processes: 1) extensive acute multifocal, predominantly perivascular hemorrhages, bilaterally distributed in cerebral white matter and subcortical nuclei and less severe involvement of the midbrain, but relative or complete sparing of the middle and lower brainstem and cerebellum, ie, structures supplied by posterior circulation vessels, as well as the germinal matrix, ventricles, and choroid plexus (Figure 1); 2) severe diffuse microgliosis with prominent involvement of white matter, subcortical nuclei, and cerebral cortex; and 3) acute to subacute hypoxic-ischemic and metabolic encephalopathy.

The extensive acute hemorrhages in basal ganglia and white matter had mainly perivascular distributions. In many areas, focal attenuation or disruption of vessel and endothelial cell apoptosis or necrosis were associated with transmural egress of erythrocytes (Figure 2;

available at www.jpeds.com). Diffuse cerebral white matter and subcortical nuclear microgliosis was associated with edema (Figure 3; available at www.jpeds.com). Acute hypoxic-ischemic-metabolic encephalopathy was associated with diffuse pericellular and microvesicular edema, increased metabolic (Type 2) astrocytes, apoptosis or nuclear pyknosis of oligodendrocytes, and cytoplasmic eosinophilia and nuclear pyknosis in neurons (Figure 4; available at www.jpeds.com). Related subacute injury was associated with loss of neurons, proliferation of reactive hypertrophic astrocytes, re-endothelialization of previously damaged ependymal linings, and gliotic micro-infarcts (Figure 4).

Immunohistochemical staining revealed extensive glial fibrillary acidic protein (GFAP) immunoreactivity in central white matter, and moderate labeling of periventricular white matter and germinal matrix zones (Figure 5, AD). GFAP immunoreactivity reflects gliosis that would have taken longer than 24 hours to become established. Immunostaining for CD34, which detects endothelial cells, demonstrated markedly reduced labeling of vessels surrounded by recent hemorrhage, and robust labeling of intact vessels that were unassociated with hemorrhage (Figure 5, IL). CD68 immunostaining demonstrated diffuse infiltration of white matter and subcortical nuclei by individual, small clusters, or large aggregates of macrophages/microglia (Figure 5, E-H).

Discussion

Pregnancy with type 1 diabetes mellitus is associated with obstetric complications and increased risk of congenital malformations, perinatal mortality, and neonatal morbidity^{19–24}. High rates of fetal morbidity and mortality occur with maternal ketoacidosis²⁵ due to rapid transfer of ketoacids and glucose across the placenta^{26, 27}. Diabetic ketoacidosis-induced fetal distress is mediated by: 1) decreased uteroplacental blood flow due to osmotic diuresis and attendant volume depletion; 2) maternal acidosis driving fetal acidosis and electrolyte imbalance; 3) maternal hypokalemia promoting fetal hypokalemia, myocardial suppression and arrhythmias; 4) maternal hypophosphatemia- associated reductions in 2,3-diphosphoglycerate, leading to impaired oxygen delivery to the fetus; or 5) fetal hyperglycemia and hyperinsulinemia which can cause pathophysiological increases in oxidative metabolism, and culminate paradoxically in fetal hypoxemia^{28–31}.

In a study of maternal type I diabetes and ketoacidosis, better pregnancy outcomes, i.e. infant survival, was associated with lower gestational age, serum glucose, and insulin requirement, and shorter duration of unresolved metabolic imbalances, whereas high rates (57%) of fetal demise were linked to new onset of type 1 diabetes³². Failure of postmortem examinations to identify anatomical causes of stillbirth suggest pathophysiological processes related to ketoacidosis and dysregulated metabolism mediate fetal demise. Similarly, in the present case, the general autopsy showed evidence of multiple organ failure syndrome rather than a specific cause of death. Because multiple organ failure syndrome can be associated with generalized inflammatory responses to pro-inflammatory cytokines³³, in the present case, new onset Type 1 diabetes could have provoked the fetal inflammatory responses that led to multiple organ failure syndrome^{17,18}.

The uniqueness of this case pivots on the striking neuropathological abnormalities including: extensive acute, predominantly perivascular hemorrhages throughout cerebral white matter and subcortical nuclei, with a rostral-to-caudal decreasing gradient in severity toward the brainstem and spinal cord, and with sparing of the cerebellum; and severe diffuse microgliosis with abundant distributions in cerebral gray and white matter structures. Extensive acute hemorrhage was associated with degeneration or necrosis of medium-size vessel walls, likely corresponding to effects of injury, possibly inflammatory in nature. The hemorrhage epicenters were distributed in central white matter and subcortical nuclei rather than germinal matrix/periventricular zones as commonly occur in the same age group of premature infants ³⁴ Because the hemorrhagic lesions emerged in the terminal postnatal clinical course, they most likely were consequences of the pathophysiologic factors mediating vasculopathy in the brain. The diffuse microgliosis, together with the increased abundance of CD68 macrophages and extensive GFAP-positive gliosis suggests that neuroinflammatory processes mediated the extensive parenchymal injury, including vasculopathy with attendant hemorrhage. We hypothesize that maternal diabetic ketoacidosis produced an inflammatory milieu that set the stage for multiple organ failure, brain metabolic dysfunction and cerebral vasculopathy. Mechanistically, ketone bodies, specifically acetoacetate and beta- hydroxybutyrate, which accumulate during ketoacidosis, function as pro-inflammatory agents that injure endothelial cells, including those of the blood-brain barrier ³⁵ In addition, maternal-driven fetal hyperglycemia may have contributed to fetal and neonatal inflammation via increased generation of glucose metabolites and reactive oxygen species, or exacerbated effects of hypoxia-ischemia to the brain ³⁶.

Pro-inflammatory cytokine exposures can worsen brain injury following perinatal insults ³⁷ C-reactive protein (CRP), interleukin-6 (IL-6), IL-1 β , and tumor necrosis factor- α (TNF- α) are well recognized inflammatory mediators of endothelial cell injury. CRP levels are elevated in severe diabetic ketoacidosis and in the early stages of insulin treatment ^{38, 39}. CRP stimulates macrophage production of cytokines such as IL-6, IL-1 β , and TNF- α and thereby increases endothelial cell permeability and vasogenic edema. Concerning the current case, we speculate that combined effects of increased vascular permeability and neuroinflammation caused extensive cerebral hemorrhage and edema, which were the main factors contributing to demise of this infant.

Although the mechanisms of cerebral edema in the setting of diabetic ketoacidosis are not fully understood, consideration has been given to the role of accumulated intracellular osmoles, such as taurine, myoinositol, acetoacetate, and ketones ^{40, 41}. Rapid declines in serum osmolality can cause brain cells to experience relative hyperosmolarity due to slow dissipation of intracellular osmolytes ⁴². Furthermore, hyperglycemia, ketosis, acidosis, ischemia, and insulin administration can cause vasogenic cerebral edema due to increased intracellular ion concentrations and attendant glial swelling, disruption of endothelial cell function, and compromised integrity of the blood-brain barrier ⁴³.

The onset of Type 1 diabetes during pregnancy is a highly unusual event. Type 1 diabetes, like other autoimmune diseases, can result in trans-placental passage of IgG auto-antibodies and inflammatory mediators that cause fetal or neonatal injury ^{44, 45}, including CNS vasculopathy, encephalopathy, or neurotoxicity ^{46, 47}, and result in long- lasting

developmental deficits⁴⁸. Pregnancy-associated maternal Type 1 diabetes can predispose the fetal brain to inflammation and vasculopathy in utero. In the present case, the advanced degrees and subacute nature of several lesions suggest that the neuroinflammatory injury in the central nervous system began in utero, several weeks prior to birth, and was the root cause of neonatal encephalopathy and vasculopathy leading to extensive terminal brain hemorrhage.

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Figure 1.

Macroscopic pathology of the fixed brain. (A) Cerebral hemispheres exhibiting severe edema with broadened gyri. The architectural disruption of the left occipital lobe (lower left compared with right) is due to parenchymal friability and encephalomalacia. (B) Coronal section through the frontal lobes with extensive hemorrhage throughout white matter (wm) and relative sparing of the cortex (arrow). The ballooned appearance is due to severe hydrocephalus. (C) Midbrain with extensive symmetrical hemorrhage throughout its central portion. (D) Pons with symmetrical hemorrhage restricted to the tegmentum (arrow). (E) Medulla with small hemorrhages restricted to the tegmentum (arrows). Note complete sparing of the cerebellum. The 4th ventricle (v) is patent but compressed due to edema.

Figure 2 (online).

Vasculocentric hemorrhages linked to underlying vasculopathy. (A) Frontal lobe central white matter and (B) internal capsule showing numerous discrete hemorrhages. (C, D) Higher magnification images of hemorrhages showing a paler zone where blood vessel fragments are surrounded by lympho-mononuclear inflammatory cells. Arrows show vascular remnants and fibrinoid vascular necrosis with adjacent hemorrhage caused by disruption of vessel wall integrity. Luxol Fast Blue, Hematoxylin and Eosin (LHE) stains (Original magnifications: A-20x; B-40x; C & D-400x).

Figure 3 (online).

Temporal lobe white matter edema and microgliosis. (A) Low magnification image showing diffuse hypercellularity with numerous vacuoles. (B) Higher magnification image demonstrating a region of low cellularity due to cell loss with only scattered remaining oligodendrocytes (circled), microglial cell infiltrates (irregular rod-shaped nuclei-arrows), and edema (clear large and small vacuoles). (C) Another region of white matter showing hypercellularity. (D) Higher magnification image of Panel C showing edema (v-clear vacuole), metabolic astrocytes (asterisk), and microglia (arrow). LHE staining, Original magnifications: A-100x, B-C-400x, D-650x

Figure 4 (online).

Cortical, periventricular and central white matter pathology. (A) Full thickness frontal cortex demonstrating normal organization for gestational age. The boxes correspond to higher magnification images in the indicated panels. (B) Hypercellularity with microgliosis of the molecular layer of cortex. (C) Mild edema but generally well-preserved cellularity. (D) Pronounced pericellular edema in deepest layer of cortex. (E) Frontal- Subcortical white matter (subjacent to region shown in Panel D) infiltrated by microglia and lymphomononuclear cells. (F) Parietal- The normally delicate single-layered ependymal lining has been replaced by multilayered proliferated ependymal and glial cells (double arrow), reflecting a subacute response to injury. The underlying parenchyma is relatively hypocellular, edematous, and has abundant metabolic astrocytes. Note cellular debris [D] in ventricular cavity shown at the upper right. (G, H) Occipital-Dense chronic inflammatory reaction (large circle) in the subventricular zone with loss of the normal architecture and

parenchymal destruction. Boxed area is magnified in Panel H. (H) Higher magnification image of densely cellular zone G showing variability in nuclear size; larger pale nuclei belong to astrocytes whereas the smaller nuclei with dense chromatin predominantly correspond to lymphocytes and microglia. Original magnifications: A, 20x; B-E, 100x; F-G, 200x; H, 400x.

Figure 5.

Adjacent histological sections of frontal lobe white matter were immunostained for (A-D) glial fibrillary acidic protein-astrocytes, (E-H) CD68-macrophages, and (I-L) CD34-vascular endothelial cells. Positive immunostaining reactions are indicated by the brown DAB precipitates. The tissue sections were counterstained with hematoxylin to reveal unlabeled cells and structures. (A) Intact, immediately subcortical white matter with mild edema. (B) Reactive astrocytes (gliosis) in subcortical white matter with recent perivascular hemorrhage and inflammation (nucleated cellular infiltrate). (C) Moderate and (D) severe reactive gliosis associated with inflammatory infiltrates in periventricular white matter. (E-H) CD68-immunoreactive macrophages were observed (E) intravascular, (F) in clusters adjacent to hemorrhage, (G) distributed in edematous white matter without hemorrhage, and (H) within vessels exhibiting vasculopathy and fibrinoid necrosis of the wall. (I-L) CD34. I) Normal intact white matter vessel with endothelial cell labeling throughout its entire cross section. (J-L) White matter vessels with surrounding hemorrhage showing gaps in CD34 immunoreactivity (arrows). Panel J shows abundant inflammatory cells close to the vessels with small gaps in CD34 labeling. Larger gaps in CD34 immunoreactivity were associated with larger parenchymal hemorrhages. Original magnifications: A-D and I-L, 100x; E-H, 400x.