A Practical Forensic Approach to Fatal Pediatric Endocrinopathies

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ABSTRACT

Of all of the organ systems in clinical medicine, the endocrine system is among the most difficult to master since it requires a detailed and knowledgeable integration of many of the body's separate systems. In forensic pathology, the evaluation of a sudden unexplained death can be challenging depending upon the particular disease processes and the organ systems that are affected. It is therefore not surprising that the investigation of sudden unexplained deaths involving the endocrine system can be particularly daunting. This review attempts to focus upon three of the major endocrine/metabolic conditions that may be potentially life-threatening and fatal in the pediatric population — hypoglycemia, adrenal insufficiency, and hyperthyroidism — and to provide forensic pathologists with a practical strategy for working up these cases. By adopting a more focused and selective approach to pediatric endocrine conditions rather than an exhaustive and comprehensive study of them, it is the intention of this review to make these disorders more manageable conceptually and to optimize the chance of arriving at a decisive and ultimately accurate postmortem diagnosis. *Acad Forensic Pathol.* 2016 6(2): 258-270

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INTRODUCTION

The evaluation of a sudden, unexpected, and unexplained death in an infant, child, or teenager is challenging enough for a forensic pathologist. Add to this the inherent nonspecificity of clinical presentation and it quickly becomes apparent why the diagnosis of fatal pediatric endocrine or metabolic disorders represents among the most difficult of postmortem diagnostic workups. However, it is certainly understandable and therefore should not be at all surprising that the clinical (antemortem) assessment of endocrine diseases is no less challenging in the postmortem setting. It is hoped that by taking a pragmatic approach toward the workup of fatal pediatric endocrinopathies, a clear and efficient evaluation of these conditions will emerge.

DISCUSSION

How Does a Life-Threatening Pediatric Endocrine/Metabolic Emergency Present Clinically?

As mentioned above, the nonspecific nature of endocrine and metabolic derangements makes the clinical index of suspicion critically important in diagnosing these conditions. But what are the clues to formulate such an index of suspicion? The following is a short summary of clinical indicators:

1. **Altered mental status:** The spectrum of central nervous system (CNS) impairment can range from mild drowsiness to irritability, lethargy, frank obtundation, stupor, and coma. Any noticeable change in mental status should always prompt a consideration of an endocrine or metabolic disturbance, particularly in infants and young children who are unable to describe their symptoms in more detail.
2. **Additional neurologic signs:** In addition to the above-listed signs of altered sensorium, other clinical manifestations referable to the central nervous system include tremors, seizure activity, abnormal muscle tone (hypo- and hypertonia [hypo-/hyperreflexia]), spasticity, and irregular breathing patterns (e.g., irregular, shallow [Cheyne-Stokes] respirations).

3. **General metabolic effects:** These can be as wide-ranging as the Kussmaul respirations related to diabetic ketoacidosis and as nonspecific as signs of nausea, vomiting, poor feeding, and weight loss (failure to thrive).
4. **Cardiovascular effects:** A clinical presentation that includes any combination of the signs of tachycardia, hypotension, and frank shock should also raise the consideration of a potentially life-threatening endocrine or metabolic condition.

Therefore, the differential diagnosis of any of the above indicators presenting in a neonate, infant, toddler, child, or adolescent should always include endocrine and metabolic disease processes (1-5).

Which Laboratory Specimens Should be Collected for a Suspected Fatal Pediatric Endocrine/Metabolic Disorder?

In a living patient, samples obtained from the patient at a given point in time are divided into two main types: 1) initial (critical) samples and 2) archival samples. Initial/critical samples in a living pediatric patient are defined as those obtained shortly after the infant or child comes to the emergency department or intensive care unit and intravenous access has been obtained. They may encompass any laboratory test performed for purposes of assessing the overall fluid, electrolyte, and metabolic balance and for formulating a practical differential diagnosis, including serum electrolytes (sodium, chloride, potassium, calcium, phosphate, and magnesium), glucose, bicarbonate, urea nitrogen, and creatinine; blood gases; and urinalysis. Archival samples, while obtained at the same time as the initial samples, are stored in the laboratory while the initial samples are being tested so that the results of these initial tests can most effectively direct the pediatric practitioner toward a specific diagnosis and corresponding treatment. Examples of endocrine laboratory determinations performed on archival samples include assays for serum insulin, cortisol, adrenocorticotrophic hormone (ACTH), 17-hydroxyprogesterone, thyroxine (T_4), triiodothyronine (T_3), thyroid-stimulating hormone (TSH), growth hormone (GH), parathyroid hormone (PTH), and 1,25-dihydroxyvitamin D (1-3).

In the postmortem forensic setting, the effective acquisition, testing, and interpretation of archival samples is made more difficult due to several key factors. To begin with, postmortem chemical changes may threaten the stability of a potential analyte and render an assay uninterpretable. Another important restriction inherent in a postmortem laboratory investigation is the requirement for interpretation of a single result as opposed to a longitudinal comparison of values to evaluate trends (6). Lastly, there is no feasible way to perform provocative testing in the postmortem situation (e.g., the administration of glucagon in hypoglycemic states to assess the adequacy of hepatic glycogen stores).

However, as pessimistic as the above considerations appear, there are some ways to work with these limitations. Postmortem degradation can be minimized by drawing a sample as soon as possible after death, even the day before the autopsy is performed. The sample can be centrifuged and the serum frozen; this will optimize the stability of the specimen. Secondly, as was illustrated effectively by Clapper et al., postmortem measurements can be combined or integrated with antemortem determinations to establish a sequence, pattern, or trend; these authors used both antemortem and postmortem serum cortisol determinations to establish a diagnosis of acute adrenal insufficiency by demonstrating a precipitous decline of postoperative antemortem cortisol values and an even lower postmortem concentration (6). Also, immunohistochemical studies can be used to assess at least semiquantitatively the amount of residual or reserve endocrine component within a particular tissue. Perhaps most significantly, with the advent of molecular genetic diagnosis, the opportunity for postmortem testing has been substantially expanded and enhanced.

The following is a guideline for postmortem testing in infants and children whose deaths are suspected to be the result of endocrine and metabolic derangements. This review will attempt to focus on the most common pediatric endocrine/metabolic emergencies that culminate in death (i.e., fatal pediatric endocrinopathies), including hypoglycemia, adrenal insufficiency, and hyperthyroidism. These are summarized in **Tables 1 and 2**.

Hypoglycemia

Hypoglycemia is a potentially life-threatening condition by virtue of the fact that, unlike other tissues that have the capability to utilize ketones as an alternate energy source, in the absence of glucose, the brain does not have a substitute energy source, and therefore, has an absolute and unconditional requirement for glucose. Hypoglycemia sustained to a critical degree and duration has the potential to progress to neuroglycopenia, with irreversible deleterious effects on the brain and ultimately the vital centers. The maintenance of physiologic blood glucose levels is dependent upon the proper balance between hepatic glucose production and the utilization of glucose by the body. In turn, glucose utilization by most of the tissues of the body is determined by both insulin and the ability of those tissues to use ketones as an alternative energy source in the absence of glucose. Therefore, derangements caused by hypersecretion of insulin or by the inability to generate sufficient numbers of ketone bodies, such as in fatty acid oxidation disorders, result ultimately in increased glucose utilization and an absence of ketones from the blood or urine. The presence of ketones is associated with conditions in which glucose utilization by the tissues exceeds hepatic glucose production but the counterregulatory hormones normally secreted to stimulate glycogenolysis and gluconeogenesis in order to maintain euglycemia – namely, glucagon, epinephrine, cortisol, and growth hormone, or the enzymes and substrates involved in the glycogenolysis and gluconeogenesis processes themselves – are insufficient (1-5).

The paradox, and ultimate dilemma, of the postmortem diagnosis of hypoglycemia (defined clinically in pediatrics as a plasma glucose concentration of <45 mg/dL in a symptomatic infant or child, or <35 mg/dL in an asymptomatic neonate) is that it is essentially a condition that is undiagnosable postmortem. This is because the methods utilized to assay blood glucose in the antemortem state are not adaptable to postmortem situations. Given that antemortem determinations of electrolytes and glucose in the serum cannot be extrapolated to postmortem assays because

Table 1: Summary of Etiologies and Clinical Features of the Most Common Fatal Pediatric Endocrinopathies

Etiologies/Differential Diagnosis	Clinical Manifestations
<p>Hypoglycemia</p> <p>*Infants (birth to six months of age):</p> <ul style="list-style-type: none"> - Transient hyperinsulinism (small for gestational age; infants of diabetic mothers; Beckwith-Wiedemann syndrome) - Congenital hyperinsulinism (HI) - Fatty acid oxidation (FAO) defects: acylcarnitine, acyl-CoA dehydrogenase deficiencies - Infection/sepsis - Defective glycogenolysis (glycogen storage diseases, galactosemia; insufficient glycogen stores; growth hormone [GH] deficiency) - Defective gluconeogenesis (galactosemia, hereditary fructose intolerance, fructose-1,6-biphosphatase deficiency; GH, cortisol deficiencies; organic or aminoacidurias; alcohol (ethanol) or salicylate intoxication) - Hepatic failure (multifactorial) <p>*Children (>6 months of age):</p> <ul style="list-style-type: none"> - Drugs (insulin; oral hypoglycemic agents; salicylates; ethanol) - Infection/sepsis - Late-presenting FAO disorders and organic or aminoacidurias; - Ketotic hypoglycemia (rarely fatal) - Malnutrition (anorexia/bulimia) - Hepatic failure 	<p>*Neonates and infants</p> <ul style="list-style-type: none"> - Altered mental status (lethargy, irritability, jitteriness, coma, seizures) - Apnea <p>*Children</p> <ul style="list-style-type: none"> - Autonomic/adrenergic: tachycardia, sweating, cold extremities, anxiety, weakness, hunger, paresthesias - Central nervous system/neuroglycopenic: headache, irritability, confusion, fatigue, abnormal/bizarre behavior, amnesia, blurred vision, diplopia, coma, seizures
<p>Adrenal Insufficiency</p> <ul style="list-style-type: none"> - Congenital adrenal hyperplasia (CAH): 21-hydroxylase, 11-hydroxylase, 3-β-ol dehydrogenase, 17-hydroxylase/desmase, steroidogenic acute regulatory protein (STAR) - Autoimmune (Addison disease) - Adrenal infections (tuberculosis, fungal) - Adrenal hemorrhage (trauma, shock; Waterhouse-Friderichsen syndrome) - Suppression of hypothalamic-pituitary-adrenal axis by exogenous glucocorticoids - Congenital adrenal hypoplasia (X-linked and autosomal recessive forms) - Hypothalamic/pituitary causes: empty sella syndrome, septo-optic dysplasia, tumors, trauma, radiation, infections 	<ul style="list-style-type: none"> - Weakness, anorexia, vomiting, weight loss, salt craving - Hyperpigmentation of skin and mucous membranes (gingivae) - Tachycardia, hypotension, pallor, poor peripheral perfusion, cool/clammy skin - Altered mental status, potentially progressing to coma - Dehydration (sunken eyes, dry mucous membranes, loss of normal skin turgor) - In female infants: clitoromegaly, ambiguous genitalia
<p>Hyperthyroidism</p> <ul style="list-style-type: none"> - Graves disease (including release of stored thyroid hormone following iodine [I 131] treatment) - Neonatal Graves disease (transient congenital hyperthyroidism) - Hashimoto thyroiditis - Functional ("toxic") thyroid adenoma - McCune-Albright syndrome - Excessive intake of exogenous thyroid hormone (in hypothyroid child or as toxic ingestion in a euthyroid child) 	<p>*Neuropsychiatric</p> <ul style="list-style-type: none"> - nervousness, agitation, difficulty concentrating, delirium, psychosis, headache, hyperreflexia <p>*Constitutional/metabolic/endocrine</p> <ul style="list-style-type: none"> - Heat intolerance, weight loss, failure to thrive (infants), hair/skin changes, menstrual irregularities <p>*Gastrointestinal</p> <ul style="list-style-type: none"> - Diarrhea <p>*Ocular</p> <ul style="list-style-type: none"> - Lid lag, exophthalmos <p>*Cardiovascular</p> <ul style="list-style-type: none"> - Palpitations, dyspnea, tachycardia, widened pulse pressure, systolic hypertension, tachypnea, rales <p>*Localized (to thyroid gland)</p> <ul style="list-style-type: none"> - Diffuse goiter, nodular goiter, solitary nodule

of the unpredictable effects of postmortem hemolysis, clotting, glycolysis, and glycogenolysis, forensic pathologists have adopted the vitreous humor as a surrogate matrix for such determinations. The problem with respect to glucose, however, is that vitreous glucose decreases unpredictably and erratically due to continued metabolism, and may reach a value of <10 mg/dL within several hours after death. Therefore, since a postmortem diagnosis of hypoglycemia is still not achievable with the current state of postmortem chemistries, the most realistic alternative is to use an antemortem glucose value and to attempt to work it up based upon the background clinical information that is available (7).

The clinical presentation of hypoglycemia is largely age-dependent. Classically, in adults and children, two categories of signs are manifested: 1) those related to the sympathetic autonomic nervous system (i.e., adrenergic), such as sweating, tachycardia, cold extremities, anxiety, and weakness; and 2) those referable to the central nervous system (i.e., neuroglycopenic), namely, headache, irritability, confusion, fatigue, abnormal behavior, amnesia, altered mental status, visual disturbances, coma, and seizures. However, in newborns and young infants, the signs may be as nonspecific as poor feeding, irritability, jitteriness, and lethargy, progressing unpredictably to coma or seizure activity.

Table 2: Summary of Postmortem Laboratory and Molecular Genetic Workup of Most Common Fatal Pediatric Endocrinopathies

Condition	Pertinent Laboratory Specimens and/or Findings (Postmortem/Antemortem)	Molecular Genetic Tests
Hypoglycemia	<ul style="list-style-type: none"> -Point of care glucose -Serum glucose -Electrolytes -Urinalysis (U/A) including ketones *Archival sample(s): <ul style="list-style-type: none"> - Insulin - C-peptide - Lactate - Ketones - Cortisol - Toxicologic examination for ethanol, salicylates, sulfonylureas 	Congenital hyperinsulinism (HI) <ul style="list-style-type: none"> - K_{ATP} channel genes: <i>ABCC8</i>, <i>KCNJ11</i> - <i>GCK</i>, <i>GLUD1</i>, <i>HADH1</i>, <i>UCP2</i>, <i>HNF4A</i>, <i>HNF1A</i>, <i>SLC16A1</i>, <i>HK1</i>, <i>PGM</i>
Adrenal insufficiency	<ul style="list-style-type: none"> - Electrolytes and glucose (hyponatremia, hyperkalemia, hypoglycemia, normal anion-gap metabolic acidosis) *Archival sample(s): <ul style="list-style-type: none"> - Serum cortisol - Serum adrenocorticotrophic hormone - Plasma renin activity - 17-hydroxyprogesterone - Antiadrenal antibodies 	Congenital adrenal hyperplasia (CAH) <ul style="list-style-type: none"> - <i>CYP2A21</i> (>90%) - <i>CYP11B1</i>, <i>HSD3B2</i>, <i>CYP17A1</i>, <i>STAR</i>, <i>CYP11A1</i>, <i>POR</i> Adrenal hypoplasia congenita <ul style="list-style-type: none"> - <i>DAX-1</i>
Hyperthyroidism	<ul style="list-style-type: none"> *Archival sample(s): <ul style="list-style-type: none"> - Thyroid hormones (free T_4, T_3) - Thyroid-stimulating hormone (TSH) - Antithyroglobulin antibodies - Antiperoxidase antibodies - Anti-TSH antibodies (thyroid-stimulating immunoglobulin [TSI]) 	None presently

The differential diagnosis of a hypoglycemic infant or child who has died is understandably broad and includes conditions related to increased glucose utilization as well as decreased hepatic glucose production. Additionally, the specific etiologies of hyperglycemia are also age-dependent. From birth to approximately six months of age, hyperinsulinism is the most common explanation for increased glucose utilization. The increased insulin activity may be either transient or persistent. Examples of transient conditions include premature and small for gestational age babies, who have both immature enzymatic apparatus and reduced substrate in the form of limited glycogen stores and protein mass that predispose to insufficient hepatic glucose production. Other causes of transient hypoglycemia are gestational or nongestational maternal diabetes (infants of diabetic mothers). Probably the conditions that would be deemed most potentially life-threatening are the persistent hyperinsulinemias, which may encompass the Beckwith-Wiedemann syndrome, focal pancreatic islet cell hyperplasia/ad-enomatosis, and diffuse islet cell hyperplasia due to mutations in the K^+ -ATP channel genes (*ABCC8* and *KCNJ11*). The other important category of diseases to consider during this age range is the fatty acid oxidation (FAO) disorders such as acylcarnitine deficiency and the acyl-CoA dehydrogenase deficiencies (small-, medium-, and large-chain types [LCAD, MCAD, and SCAD]). Inherited and acquired defects in glycogenolysis (such as glycogen storage diseases and galactosemia, and growth hormone deficiency) and gluconeogenesis (including galactosemia, hereditary fructose intolerance, fructose-1,6-diphosphatase deficiency, ethanol or salicylate intoxication, inborn organic or aminoacidurias, hormone deficiencies, and liver failure) also account for causes of hypoglycemia in the newborn and infant period (1-5).

In children and adolescents, a different yet slightly overlapping group of entities forms the differential diagnosis. Causes of increased glucose utilization include drugs, most commonly insulin and oral hypoglycemic agents (sulfonylureas), late-presenting FAO disorders, and infection/sepsis. Entities responsible for decreased hepatic glucose production consist of ketotic hypoglycemia, the most frequent cause of hypogly-

cemia in children, ascribed to a reduced muscle mass that undermines the body's ability to utilize amino acids as substrates for gluconeogenesis; late-presenting organic or aminoacidurias; malnutrition, anorexia, and bulimia; liver failure; and exogenous agents such as ethanol or salicylates. Finally, infection and sepsis are known causes of increased glucose utilization and depletion and of impaired hepatic glucose production. A rare cause of hypoglycemia that should not be forgotten is an insulinoma (insulin-secreting tumor of the β pancreatic islet cells) (2-5).

One of the greatest dangers inherent in a review of this type is to present what are essentially "laundry lists" of differential diagnoses (for the various potentially life-threatening or fatal endocrinopathies). Therefore, the challenge for an audience of forensic pathologists is to sustain the critical thinking process in the workup. One way to accomplish this is through a diagnostic algorithm. Dividing hypoglycemia into ketotic and non-ketotic types allows for differentiation into a shorter list of diagnostic possibilities. This can be done quite simply in the forensic laboratory by testing the urine for the presence of ketones. Thus, ketonuria would indicate considerations of ketotic hypoglycemia, adrenal insufficiency, panhypopituitarism (growth hormone deficiency), other inborn errors of metabolism (i.e., organic acidemia, mitochondriopathies, galactosemia, and hereditary fructose intolerance), and a fasting/starvation state. Absence of ketones in the urine generates a list that includes hyperinsulinemic states and FAO defects (4, 8).

Postmortem Laboratory Evaluation and Molecular Genetic Testing in Cases of Suspected Fatal Hypoglycemia

The laboratory workup of cases of suspected fatal hypoglycemia in the pediatric population is limited primarily to insulin and C-peptide determinations. To review briefly, insulin is synthesized as a long peptide chain precursor (proinsulin) composed of three chains, namely, A and B, which are connected by a third peptide chain (C-peptide). In response to a hyperglycemic stimulus, proinsulin is converted to active insulin by means of enzymatic cleavage, which liberates the

C-peptide from the A and B chains that are now linked by two disulfide bridges. Because each molecule of insulin is originally accompanied by C-peptide, the insulin and C-peptide are actually secreted in equimolar amounts. Therefore, their ratio in the blood should theoretically approach unity. The forensic significance of this ratio lies in the fact that C-peptide is unique to endogenous insulin and is not present in either human or animal exogenous insulin; thus, a high ratio of insulin to C-peptide (I/C) suggests an exogenous origin of the insulin, while an I/C ratio close to one (and potentially slightly lower given the slightly longer half-life of C-peptide than that of insulin) would be expected in an endogenous source of insulin (such as an insulinoma) or an exogenous non-insulin hypoglycemic agent (e.g., sulfonylurea, which is also detectable in the plasma and urine) (7, 9, 10).

Unfortunately, these two determinations only address a small fraction of the total number of causes of hyperinsulinemic hypoglycemia. Therefore, molecular genetic testing is essential to follow up these other etiologies. The most common and clinically severe of the monogenic forms of congenital hyperinsulinism (HI) is caused by inactivating mutations of the genes of the two subunits comprising the K_{ATP} channel: the sulfonylurea receptor (SUR1) encoded by the *ABCC8* gene and the inward-rectifying potassium channel (Kir6.2) encoded by the *KCNJ11* gene. Mutations in these genes collectively comprise 40-45% of all cases of HI and approximately 82% of diazoxide-unresponsive patients (diazoxide, being a K_{ATP} channel agonist, is ineffectual when the defect in *ABCC8* or *KCNJ11* eliminates the function of this channel). Additional mutations have now been identified on nine other genes encoding enzymes, transporters, and transcription factors orchestrating insulin secretion and accounting for approximately 5-10% of cases of HI: the *GCK* gene encoding glucokinase; the *GLUD1* gene encoding glutamate dehydrogenase; the *HADH1* gene encoding short-chain L-3-hydroxyacyldehydrogenase (SCHAD); the *UCP2* gene encoding the UCP2 protein; the *HNF4A* and *HNF1A* genes encoding the hepatocyte nuclear factors 4 and 1; the *SLC16A1* gene encoding the MCT1 (pyruvate transporter); the *HK1* gene encoding hexokinase-1; and the *PGM1* gene en-

coding phosphoglucomutase-1 (11, 12). The genetic basis for the remaining 45-55% of patients with HI remains elusive. Currently, a comprehensive familial hyperinsulinism panel targeting ten genes is available through one genetics laboratory in the United States and a second targeting seven genes through a laboratory in Frankfurt, Germany.

Differential Diagnosis of Hyperinsulinism

Included in the differential diagnosis of congenital HI (which encompasses both isolated and syndromic forms) are several other conditions of which the forensic pathologist should be aware. These include most commonly drug-induced hyperinsulinemic hypoglycemia secondary to oral hypoglycemic/antidiabetic agents, β -blockers, antiarrhythmic drugs, and Munchausen syndrome by proxy in the form of insulin injection or sulfonylurea administration; and insulinoma, with an estimated incidence of one in one million adults but even rarer in children (the youngest patient reported in a 1991 review was eight years old) (3-5, 8-12).

Adrenal Insufficiency

Adrenal insufficiency becomes life-threatening and potentially fatal when the glucocorticoids and mineralocorticoids secreted by the adrenal cortical cells are unable to meet metabolic demands that normally allow the body to maintain physiologic amounts of total body sodium and water, blood glucose concentrations, and hemodynamic stability under conditions of acute stress. Normally, the adrenal cortex has two mechanisms by which to maintain homeostasis in stressful conditions, which are accomplished by the secretion of glucocorticoids and mineralocorticoids. In the case of the former, a stimulus of stress or hypoglycemia induces the hypothalamus to secrete corticotropin-releasing factor (CRF), which in turn, stimulates the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland, thereby acting on the zona fasciculata cells within the adrenal cortex, which secrete cortisol, a glucocorticoid that constitutes one of the major defenses against hypoglycemia and shock. In the latter, a primary stimulus of decreased effective

circulating blood volume activates the renin-angiotensin system, leading to the stimulation of the adrenal cortical zona glomerulosa cells to produce aldosterone, in concert with the stimulus of elevated serum potassium (hyperkalemia). Aldosterone is the major mineralocorticoid that functions to retain sodium and water and excrete potassium and hydrogen ions by acting upon the distal renal tubular epithelial cells, thereby maintaining total body water and sodium and protecting the body from dehydration, hyperkalemia, and potentially catastrophic shock. Full-blown adrenal (cortical) insufficiency, therefore, is an emergency because it is essentially a shock state that must be addressed and managed before irreversible hemodynamic collapse and organ failure ensues (1-5, 13-18).

The clinical manifestations of adrenal insufficiency reflect the above-described physiologic derangements imposed by the shock state. As a result, affected infants and children may present with weakness, anorexia, vomiting, weight loss, salt craving, and hyperpigmentation involving both skin and mucous membranes, particularly the nonexposed regions of skin, areolae, flexion creases, scars, and gingiva (a function of the melanocyte-stimulating properties of the ACTH precursor, pro-opiomelanocortin). Physical signs include tachycardia and other features of shock, such as hypotension, pallor, poor peripheral perfusion, cool and clammy skin, and altered mental status, progressing to coma. Dehydration may manifest as sunken eyes, dry mucous membranes, and loss of normal skin turgor with tenting of skin. A physical feature unique to female infants is the presence of clitoromegaly and even ambiguous genitalia ascribable to the virilizing effects of the enzymatic block in steroidogenesis with shunting of the biosynthetic pathway toward androgen production. For this reason, male infants are actually more vulnerable to life-threatening adrenal insufficiency because the absence of physical findings may delay the diagnosis (2-5).

The causes of adrenal insufficiency are myriad and include both central (hypothalamic/pituitary) and end-organ (adrenal) processes but like other endocrine disorders are largely age-dependent. As a result, primary adrenal insufficiency presenting in the newborn

period should prompt consideration of one of the conditions comprising the spectrum of congenital adrenal hyperplasia (CAH), the majority of which are inherited in an autosomal recessive fashion and are linked by the inability to synthesize appropriate (physiologic) amounts of cortisol. If mineralocorticoid (aldosterone) synthesis is also deficient, a salt-wasting tendency becomes part of the clinical picture, and if the block in the steroidogenic biosynthetic pathway diverts production toward androgen synthesis, virilizing features are present. The most common cause of CAH, accounting for approximately 95% of cases, is 21-hydroxylase deficiency, which presents as a salt-wasting (salt-losing), virilizing form of CAH. The other enzyme deficiencies, collectively comprising up to 5% of the remaining forms of CAH, include 11-hydroxylase deficiency (~4%; salt-retaining and virilizing), 3- β -ol dehydrogenase (~1%; salt-wasting and undervirilizing), 17-hydroxylase/desmase (~1%; salt-retaining and undervirilizing); and deficiency of steroid acute regulatory protein (<1%; salt-wasting and undervirilizing). One central cause of adrenal insufficiency presenting in infancy is septo-optic dysplasia, which may manifest with variable combinations of dysmorphic features, including cleft palate, syndactyly, ear deformities, and hypertelorism. Later in childhood, the most common etiology for adrenal insufficiency is suppression of the hypothalamic-pituitary-adrenal axis by exogenous glucocorticoids in steroid-dependent conditions, such as bronchial asthma, autoimmune diseases, and hypopituitarism, exacerbated by failure to augment the glucocorticoid dose during times of stress, most frequently in the form of trauma, surgery, or infection. It is important to note that when glucocorticoids are administered for a period exceeding 10-14 days, endogenous ACTH secretion may be suppressed for several weeks, thereby placing the child at risk for an adrenal insufficiency (Addisonian) crisis. After exogenous suppression, the next most frequent cause of adrenal insufficiency is autoimmune-mediated injury (Addison disease), which may be associated with additional autoimmune conditions, such as type 1 diabetes mellitus and Hashimoto thyroiditis. Less common but nevertheless potentially fatal etiologies for primary adrenal insufficiency include adrenal infections (e.g., tuberculosis,

histoplasmosis), adrenal hemorrhage secondary to trauma or shock (most frequently meningococcemia with Waterhouse-Friderichsen syndrome), and even more rare, adrenal hypoplasia congenita (X-linked or autosomal recessive) and Allgrove syndrome (adrenal insufficiency, achalasia, alacrima). Additional central causes of adrenal insufficiency are tumors, trauma, radiation injury, infections, and idiopathic hypothalamic or pituitary conditions that often present with other features of hypopituitarism (1-5).

Postmortem Laboratory Evaluation and Molecular Genetic Testing in Cases of Suspected Fatal Adrenal Insufficiency

The postmortem laboratory workup of these children is necessarily derived from the antemortem results because the characteristic metabolic profile of primary adrenal insufficiency, irrespective of the specific etiology, is hyponatremia, hyperkalemia (in ~50% of pediatric cases), and normal anion gap metabolic acidosis (reduced bicarbonate with relatively increased chloride) attributable to insufficient or absent mineralocorticoid activity, along with hypoglycemia (in up to 90% of cases) secondary to decreased glucocorticoid activity. Parenthetically, it should be pointed out that the vitreous humor cannot be used in the postmortem setting to diagnose primary adrenal insufficiency since vitreous sodium decreases slowly and vitreous potassium increases unpredictably after death. These laboratory values comprise the initial, or critical sample. Depending upon the child's clinical course, the archival blood sample may or may not have been submitted for follow-up endocrine and metabolic determinations, which include plasma renin activity (particularly if the clinical presentation is salt-wasting), serum cortisol and ACTH to distinguish primary from secondary causes of adrenal insufficiency, and 17-hydroxyprogesterone in infants with suspected CAH. Occasionally, a complete adrenal metabolic profile will have been ordered in order to identify the specific enzymatic block along the steroidogenic biosynthetic pathway. Thyroid hormone (free T_4) and TSH would also be indicated if secondary adrenal insufficiency with hypopituitarism is suspected clinically (2-3, 6-19).

The limiting factor with regard to postmortem archival testing relates to the stability of the specific analytes. Postmortem serum and urine cortisol measurements are believed to be relatively reliable, although admittedly only a few studies regarding this issue have been conducted. The largest of these, which examined postmortem specimens in 35 autopsies (20 adults and 15 children or infants), demonstrated similar values in postmortem serum from both heart and femoral blood in both age groups, and furthermore, only a slight decrease in cortisol levels over the first 18 hours postmortem (6, 20). The measurement of cortisol and 17-hydroxycorticosteroids in urine remains another option. Only one study addressing the postmortem biochemistry and immunohistochemistry of ACTH has been conducted, and this study involving 162 serial forensic autopsies failed to observe a correlation between serum or cerebrospinal fluid ACTH concentrations and postmortem interval, thus implying a relative postmortem stability of ACTH. An important qualifier of this observation, however, is that all of the blood samples were immediately centrifuged to separate the sera, and the serum samples were promptly frozen at -20°C (19). The implications of these admittedly limited investigations are nevertheless optimistic, suggesting a potential use for postmortem cortisol and ACTH measurements, provided that the conditions for their analysis are optimized. In cases where autoimmune Addison disease is suspected, postmortem detection of antiadrenal autoantibodies has been documented but an archival antemortem sample would still be the specimen of choice.

Molecular genetic testing in cases of suspected fatal adrenal insufficiency does not have the same diagnostic significance as it does in the workup of hypoglycemia but can be useful in cases where hormonal testing, which relies on the precursor to product ratio corresponding to the specific enzymatic defect, is inconclusive or nondiagnostic. Since all forms of CAH are monogenic, autosomal-recessive disorders, and >90% of cases are due to mutations in the *CYP21A2* genes responsible for 21-hydroxylase deficiency, sequencing of the *CYP21A2* gene the most common testing that would be proposed, while testing for the remaining mutations (in the *CYP11B1*, *HSD3B2*, *CY*

P17A1, *STAR*, *CYP11A1*, and *POR* genes) would be reserved for rare situations. Mutations in the *DAX-1* gene would be found in cases of X-linked congenital adrenal hypoplasia (21).

One last issue that deserves mention is the advent of newborn screening for 21-hydroxylase deficiency (21OHD). While screening unequivocally has been shown to truncate the latent period to diagnosis in infants, thus allowing the detection of disease in male infants who would be susceptible to adrenal crisis at some unpredictably later point in time, it should be noted that the first-tier testing for CAH that measures 17-OHP in dried blood is accompanied by a substantial false-positive rate, particularly in premature and critically ill infants, and false-negative rate of up to 22% when mothers have been exposed prenatally to glucocorticoids. For this reason, a negative newborn genetic screen for CAH certainly does not exclude the diagnosis, and in an infant or young child with a suggestive clinical presentation, the diagnosis should be pursued vigorously (21).

In summary, metabolic crisis resulting from adrenal insufficiency, while rare, is nevertheless a real emergency situation that can end fatally if the diagnosis is missed or delayed for a critical time period so that it progresses to refractory shock. The clinical significance of sufficient glucocorticoid secretion in the setting of stress translates to the importance of including adrenal insufficiency in the differential diagnosis of an infant or child who succumbs apparently suddenly and without explanation to an acute stress (13-17, 22).

Hyperthyroidism/Thyroid Storm

Hyperthyroidism becomes a potentially life-threatening situation when the positive inotropic and chronotropic effects of thyroid hormones on the heart (including both thyroxine [T_4] and triiodothyronine [T_3]) result in tachycardia and systolic hypertension sufficient to drive the heart into a decompensated state of high-output failure. The proposed mechanism is up-regulation of β -adrenergic receptors on the myocardium (3, 5).

The principal clinical features of hyperthyroidism are dominated by neuropsychiatric and cardiovascular signs, with the most common being nervousness, hyperactivity and/or agitation, inability to concentrate, emotional lability, tremor, fatigue, palpitations, dyspnea, heat intolerance, headaches, diarrhea, excessive appetite but accompanied by weight loss and possibly failure to thrive in an infant. One important clue in a previously diagnosed hyperthyroid child whose signs and symptoms have resolved with antithyroid medications is the reemergence of the clinical manifestations suggesting discontinuation or noncompliance with the medications. Physical examination typically demonstrates tachycardia, systolic hypertension with widened pulse pressure, a systolic flow murmur, and tachypnea. Additionally, ocular findings such as lid lag and exophthalmos might also be present and the skin may be noticeably warm and dry to slightly moist (3, 5).

Although thyroid storm is a relatively rare presentation in the pediatric population, it has been documented to occur and manifests as hyperthermia, high-output heart failure with rales indicative of pulmonary edema, vomiting, and delirium potentially progressing to coma and death (3, 5, 23).

The differential diagnosis of an acute pediatric hyperthyroid crisis is relatively narrow but the list is topped by Graves disease, which on physical exam would be recorded as a diffuse firm goiter. Other entities to consider include Hashimoto thyroiditis, a functioning ("toxic") thyroid nodule, a TSH-secreting tumor (most commonly a pituitary adenoma), McCune-Albright syndrome (autonomous production of thyroid hormone due to constitutively activated polypeptide hormone receptors), and excessive exogenous intake of thyroid hormone in a hypothyroid child. Neonatal Graves disease, also referred to as transient congenital hyperthyroidism, is a condition that must be considered in any children born to mothers with Graves disease, because transplacental passage of maternal IgG TSH receptor antibodies (thyroid-stimulating immunoglobulins) is known to occur in approximately 1% of these infants. The history is especially important in this circumstance in that such infants may not exhibit

visible or palpable goiters and even mothers treated with thyroidectomy or radioablation may still harbor TSH receptor antibodies in their serum that are capable of crossing the placenta. One extrathyroidal endocrine condition that should also be part of the differential diagnosis is a catecholamine crisis precipitated by a pheochromocytoma (3, 5).

Postmortem Laboratory Evaluation and Molecular Genetic Testing in Cases of Suspected Fatal Thyroid Storm

Once hyperthyroid crisis is considered in the differential diagnosis, the postmortem laboratory workup is relatively straightforward and may be aided by archival samples obtained antemortem. Furthermore, studies examining TSH, T_4 , and T_3 in postmortem serum have demonstrated these hormones to be relatively stable and to be comparable to antemortem clinical reference ranges. Therefore, a panel that includes assays of free T_4 , T_3 , and TSH should generally be sufficient, and hormone values may be followed up with measurements of antithyroid peroxidase and antithyroglobulin antibodies and for thyroid-stimulating immunoglobulin for diagnosis of Hashimoto or Graves disease, respectively (3, 20).

Two particular clinical scenarios that should remain within the thought process of the forensic pathologist are the discontinuation of antithyroid medication in a child with Graves disease (typically methimazole since propylthiouracil [PTU] is no longer routinely prescribed given its risk of hepatotoxicity) and the excessive ingestion of exogenous levothyroxine (by means of accidental or intentional overdose). A case referred to our office involved a death due to hyperthyroidism that was precipitated by discontinuation of methimazole in an 8-year-old child, apparently as a result of confusion among multiple caretakers in the family who were not equally aware of the importance of maintaining administration of antithyroid medication (3, 20).

A case report of a massive levothyroxine ingestion by Mandel et al. in 1989 advocated a conservative approach with propranolol based upon their experience of a benign clinical course and a higher tolerance of

a single-episode ingestion of excessive amounts of thyroid hormone by a healthy (nonhypothyroid) child (24). Subsequent case reports describing massive levothyroxine ingestions have documented a similar benign clinical course in these children. In fact, in over 9000 exposures to thyroid preparations reported in 2008 to the American Association of Poison Control Centers' National Poison Data System, only three (0.033%) major adverse outcomes were reported, none of which were fatal (25). However, a 2010 case report by Majelsi et al. documented the occurrence of frank thyrotoxicosis in a 2-year-old girl who ingested an estimated 40 150 μ g levothyroxine tablets. This report was a departure from the previous literature recommending conservative management in that the authors promoted a more aggressive approach as part of the initial management, namely, gastrointestinal decontamination, including emesis. Additionally, they observed that serial T_3 measurements provided a better correlation than T_4 and TSH concentrations with the child's clinical course and they therefore recommended the using T_3 over T_4 to monitor the clinical effects of levothyroxine ingestion (23). Given the propensity of young children to ingest medications that may appear colorful and attractive and resemble candy, it behooves the forensic pathologist to be aware of the clinical course of these events (23-26).

CONCLUSION

This review was not intended to provide a comprehensive treatment all of the fatal pediatric endocrinopathies that have been experienced and/or reported by pediatricians, emergency department physicians, intensivists, or forensic pathologists. Rather, it was designed to offer an approach to such diagnoses (Table 3). One theme that emerges clearly from this review is that, analogous to the situation with living patients, endocrine and metabolic disorders can only be diagnosed if they have been considered as part of the differential diagnosis in the first place. Therefore, it is incumbent on the forensic pathologist to exercise critical thinking and maintain an index of suspicion for fatal endocrine and metabolic disease processes in infants and children who die suddenly, unexpectedly, and without apparent explanation.

Table 3: Basic Guidelines for Optimizing Quality and Stability of Postmortem Laboratory Specimens and for Diagnostic Follow-Up in Cases of Suspected Fatal Pediatric Endocrinopathies

1. Upon suspicion of a pediatric death potentially related to an endocrine or metabolic derangement, obtain blood and urine specimens as soon as possible after death and centrifuge and freeze serum samples for "archival" testing.
2. Contact the hospital laboratory to determine whether any archival antemortem samples are being stored; if not, contact the emergency department to ascertain whether any samples were drawn but not yet submitted to the laboratory before the child or infant expired. The availability of any antemortem samples will greatly facilitate any endocrine/metabolic laboratory workup.
3. Communicate with either the hospital laboratory, molecular genetics department, or reference laboratory in order to formulate a plan for submitting samples for follow-up molecular genetic testing.
4. Because the diagnosis of hypoglycemia cannot be established postmortem, molecular genetic testing for the forms of congenital hyperinsulinism (HI) becomes especially important as an adjunct to diagnosis.

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