A three-month-old girl with liver failure: How far have we reached after autopsy?

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CLINICAL PROTOCOL

History: A three-month-old female child presented with jaundice for 15 days, progressive abdominal distension for 10 days, lethargy for 5 days, black tarry stool for 2 days. There was no history of acholic stools, skin bleeds, fever, rash, diarrhea, tachypnea or seizures.

She was born second by order of birth of a non-consanguinous union with a birth weight of 2 Kg. The antenatal and perinatal period was uneventful. Her elder 3-year-old brother was developmentally normal with no similar history. She was breast-fed, which was supplemented by diluted, sugared, cow's milk, since birth.

She was admitted elsewhere for a couple of days prior to admission where she had been given IV antibiotics and a received a blood transfusion.

Clinical examination: On admission the patient was afebrile. Vitals parameters were normal. Oral mucosal bleed was noted. Anterior fontanelle measured 1 × 1 cm and was not bulging; Weight: 3.0 kg (<−3 Z-score); Length: 49 cm. (<−3 Z-score); Occipital-frontal circumference: 33.5 cm (<−3 Z-score). She had pallor, edema and icterus. There was no cyanosis, lymphadenopathy, clubbing. There was no dysmorphism, coarse features, or specific odour. On systemic examination, abdomen was soft, non-tender, distended with central, everted umbilicus. Fluid thrill was present. On palpation, liver was irregular, firm, non-tender with smooth surface, 5 cm below the right costal margin, with a span of 8 cm; spleen was firm and 2 cm below left costal margin. On evaluation of central nervous system, she was conscious and alert, fixing, and following light. Examination of chest and cardiovascular system was normal.

Investigations: Her haematological and biochemical investigations during the hospital stay are summarised in *Table 1*.

- Lipid profile(mg/dL): Triglyceride—97.3; cholesterol—231; low-density lipoprotein—75; high-density lipoprotein—46.5
- Alpha-fetoprotein - 21,000 µg/mL
- Direct Coomb's test (DCT) and glucose 6 phosphate deficiency (G6PD): Negative
- Eye: Slit Lamp and Fundus: No cataract, choroidal-retinal synaechae, retinal abnormalities
- Ultrasonography abdomen: Liver 6.9 cm; normal outline and echotexture; hepatic vein and portal vein normal; spleen: 6.3 cm; pancreas: N size and increased echogenicity; moderate ascites
- Urine Reducing substance: Nil
- Blood/Urine/Ascitic C/S: Sterile
- Venous blood gas (at admission) pH – 7.30, HCO^{3-} 17 meq/L–: Metabolic acidosis; normal lactate
- TMS/GCMS: No abnormal metabolite for aminoacidurias, organic acidurias, fatty acid oxidation defects and urea cycle defects.

Course during hospital stay

After admission the child was started on cefotaxime (for spontaneous bacterial peritonitis), octreotide and pantoprazole. Additionally, was supported with albumin infusion, vitamin K along with other fat and water soluble vitamin supplements. Upper GI endoscopy was performed which showed a Grade I esophageal varix and diffuse gastritis. Octreotide was stopped. Milk was stopped presuming galactosemia on day 2; however, after an initial improvement there was progressive worsening in coagulopathy/ ascites. No hypoglycaemia was documented during the hospital stay. Hemoglobin dropped in absence of GI bleed, and packed red blood cells were repeated twice. On day 10 of admission, in view of fever, pneumonia and worsening counts, antibiotics were upgraded to vancomycin/imipenem. Ascitic fluid was tapped almost daily due to rapid refilling. The child continued to worsen and eventually died on day 14 of admission.

Unit's diagnosis: Metabolic liver disease ? Galactosemia ?Mitochondrial hepatopathy

Discussion on clinical protocol:

Based on data and investigations available, we were dealing with a young infant with cholestatic jaundice. Cholestasis can be classified into biliary (obstructive, large extrahepatic, or small intrahepatic bile ducts) or hepatocellular (defect in membrane transport, embryogenesis, or metabolic dysfunction) in origin. The approach to such a patient would be to first try and identify whether the cause is biliary or hepatocellular.

The presence of pigmented stools, delayed – onset of jaundice and ascites (suggestive of hepatocellular injury) in a sick child suggests more likely a hepatocellular/ intrahepatic cause. The clinical pointers that lead towards a diagnosis in infants with intrahepatic cholestasis are tabulated in **Table 2.**

The presence of uncorrectable coagulopathy (INR > 2) points towards the diagnosis of pediatric acute liver failure. (1) Liver failure in an infant with early – onset ascites are important clues to the diagnosis in this child. The common causes of liver failure in an infant and their common clinical presentation is listed in **Table 3**.

If we look at this list in context of our child, Tyrosinemia is less likely, as generally the bilirubin is well below 10 mg%, AST/ALT are only mildly elevated and alpha fetoprotein is markedly elevated.(mean level 160000 microgram/decilitre) (2) Gestational alloimmune liver disease, in which maternal immunoglobulin G causes complement-dependent severe fetal liver injury and dysregulated handling of iron by the fetal liver, causing abnormal iron distribution in the body is an important cause of neonatal liver failure in an IUGR baby, however they present earlier, AST/ALT are just mildly elevated and the liver is generally shrunken. (3) Galactosemia is a possibility, however lack of improvement in INR/albumin and ascites despite 2 weeks off milk is odd.(4)The baby was exposed to sucrose, so hereditary fructose intolerance (HFI) is possible but features that do not fit in with our case is the absence of diarrhea or vomiting and normal levels of serum phosphate and uric acid.(5) Primary mitochondrial hepatopathy unless looked for can be a missed as a cause for severe liver disease in early infancy.(6) Multisystem disease may not always occur at presentation due to heterogeneous expression of genetic defect in different tissues; IUGR and moderate to marked rise of AST/ALT as in this case is well described. HLH is unlikely because of the absence of fever and cytopenias, while Niemann Pick C seems unlikely because of the small spleen size. (7) The normal lipid profile makes Wolman's disease an unlikely possibility. One would not consider CMV and other TORCH infections in such a setting as elevated TORCH titres are often a red herring in the workup of a neonate with cholestasis. Except for acquired HSV infection which has rapid deterioration, the rest of the TORCH infections rarely cause jaundice. The delayed presentation and the absence of skin lesions and other stigmata makes HSV unlikely.

Hence to sum up, the clinical possibilities are - mitochondrial hepatopathy, galactosemia and HFI.

However, it is to be remembered that all these disorders discussed above have a varied spectrum of presentation and may at times not have a "classical" presentation as discussed above and hence it is prudent to keep an "open "mind when evaluating such a child.

PATHOLOGY PROTOCOL

Partial autopsy was done in this case; externally, prosecutor noted pallor with icterus. Serous cavities: Yielded 500 mL (icteric fluid) in peritoneal cavity, 50 mL of serous fluid in pleural cavities, 15 mL of serous fluid in pericardial cavity.

Liver- Weight: 105 gm gross. Capsular and cut surface smooth with tiny nodules (<3 mm), cut surface was bile stained. (Fig. 1) Microscopy revealed hepatocytes showing pseudo acinar transformation, with micro- and macrovesicular steatosis and prominent giant cell transformation (Fig. 2). There was distorted architecture with porto-portal and porto-central bridging. Portal tracts were replaced by fibrous bands which were extending to the peri-portal areas with fibrosis also noted in perivenular zones. Focal peri-portal cholangiolar proliferation was noted. Intrahepatocytic and cholangiolar cholestasis was noted. No macroregenerative nodules seen. Extrahepatic biliary tract is within normal limits. *Lungs*- Weight: 70 gm. On gross examination were subcrepitant with focal areas of congestion. Trachea and airways did not show inspissated secretions. Micro: Occasional secondary bronchiole showed secretions, foamy macrophages identified in most of the alveoli. Cytomegalovirus inclusions were identified in pneumocytes lining the alveoli (Fig. 3A) focally. Many pigment laden macrophages were noted as well. No fungal hyphae/abscesses were seen. *Heart*- Weight: 20 gm. All chambers and valves were within normal limits. A single 8 mm abscess noted in left ventricular wall , which microscopically is composed of central necrotic material admixed with neutrophils and giant cells and few Aspergillus hyphae (Fig. 3B). *Thymus* - Weight: 2 gm. Within normal limits. Microscopic examination showed stress-induced involution with excess of Hassall's corpuscles. Adequate representation of CD3 T lymphocytes. *Bone marrow* was within normal limits.

Discussion on pathology protocol

Histologic or ultrastructural feature is almost never specific in the diagnosis of inherited liver disorders hence pathologists must rely on integrating clinical information with the biopsy features to avoid diagnostic roadblocks.

Steatosis is a common histopathologic finding in several inherited disorders affecting liver. A perturbation of mitochondrial metabolism is associated with microvesicular steatosis. Our patient had micro and macrovesicular steatosis. Galactosemia and HFI, are classically associated with diffuse macrovesicular or mixed steatosis in the newborn or infant. In galactosemia, typical liver biopsy shows extensive periportal and intra lobular fibrosis, pseudo acinar transformation and distortion of periportal vasculature. (8) All these features were identified on the biopsy of our patient. The histopathologic features of HFI mimic those of galactosemia, except that cirrhosis is usually absent. Other causes of hepatic steatosis in an infant include Wolman's disease (hepatocyte vacuolation, foamy kupffer cells, macrophages with positive lipid stains), cystic fibrosis (inspissated material within bile ducts) and alpha 1 antitrypsin deficiency (macro nodular cirrhosis, PAS-positive, diastase-resistant

hepatocyte inclusions) features of which were not seen on histopathology. (9)

Evidence of CMV and aspiration pneumonia and aspergillus myocardial abcesses were found.

Final Diagnosis

Micronodular cirrhosis (Probably consistent with galactosemia) with portal hypertension

Myocardial abscess (fungal, aspergillus): Left ventricle

The CMV inclusions and foamy macrophages (milk globules) in lungs

Open Forum

Classic galactosemia is caused by deficient activity of galactose-1-phosphate uridylyltransferase the second enzyme of the main pathway of galactose metabolism (Figure 4) and its prevalence is 1:16,000-60,000 live-births. (10) It is an autosomal recessive disorder caused by mutations in the *GALT* gene and over 300 variations have thus far been described. Mutational analysis of the GALT gene from Indian subjects has revealed heterogeneity in the structure of the gene and the presence of novel mutations. (11) Infants with classic galactosemia generally appear asymptomatic at birth. However, after a few weeks of galactose ingestion through breast or formula feeding they start developing symptoms that, if undiagnosed and untreated, may lead to early decompensation and eventually death.

This case presented with a cirrhotic liver by the age of 3 months. The most likely cause of cirrhosis at this age with micro nodular cirrhosis and absence of iron would be galactosemia. Cataract is present at diagnosis in only 30-50% infants (4). A definite diagnosis could only have been established with red blood cell enzyme studies, which could not be performed as the child had received a blood transfusion just before admission. Screening for reducing substances in urine can be informative; however, it is not sensitive or specific. If the child is on intravenous fluids, galactosuria may no longer be present, thus leading to false negatives as seen in our child who was feeding poorly on admission. A genetic work – up could not be performed because of financial constraints.

No clinical improvement on withdrawal of lactose is odd in our case as the disease has a favourable prognosis by timely introduction of a lactose free diet. However, it is possible that the delay in diagnosis and overwhelming sepsis – bacterial, fungal (aspergillus) and CMV could have led to the worsening condition and eventual demise of the child. Depressed neutrophilic function by galactose or its metabolites and deficiency of IgM bactericidal opsonic antibodies may contribute to the high incidence of sepsis. Though, bacterial sepsis like *E. Coli* is commonest, other organisms like fungal and viral infections can rarely be the culprit in these children. (12) In the series by Sarma et al. 87.5% (21/24) of the children diagnosed with galactosemia survived, with uncontrollable sepsis and/or poor compliance leading to death in 3 children.

Long term outcomes in children who survive are associated with a few complications. One of the most frequent and well-established long-term complications is cognitive impairment with IQ standard scores in the low average (85–100) to borderline-low (<85) range. Girls and women with classic galactosemia may have primary ovarian insufficiency, with an incidence above 80%. Because of their galactose-restricted diet, patients are at risk for nutritional deficiencies, particularly calcium which should be supplemented. (10)

To conclude, a cholestatic infant with ascites, coagulopathy, and/or haemolysis should raise a suspicion for galactosemia. A lactose – free diet is lifesaving. Sepsis is a known cause of early morbidity and mortality and should be managed aggressively.

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Table 1 – Laboratory investigations during the hospital stay

	Day of admission	Day 4	Day 10	Day 13
Hb (mg/dL)	8.6	11.8	6.4	
TLC (cells/mm^3)	20,100	10,400	38,600	
DLC (N/L/M/E)	27,67,2,4	52,40,6,2		
Platelets (cells/mm^3)	4.35 L	1.22 L	2.19 L	
INR	7 (after IV vitamin K)	1.3	2.05	1.3
Fibrinogen			1.23	
Na/K/CI (mmol/L)	131/6.3/102			
Ur/Creat (IU/ml)	22/0.25		10/0.27	
Bil: T/D (mg/dL)	17.6/15	18.5/12.7	20.9/10	
AST (IU/ml)	893	1062	811	
ALT (IU/ml)	223	863	633	
ALP (IU/ml)			465	
TP: Albumin(mg/dL)	4/2.6	3.5/2	<3.5/2.1	
Ca/P/UricA (IU/ml)		9.1/3/3		
Ascitic protein	0.3 gm%		0.3 gm%	
Ascitic Fluid cells	1000 polys		Nil	
Serum Albumin Ascitic Gradient	2.6			
Ascitic Bilirubin	2 mg%			

Table 2 – Clinical clues of intrahepatic causes of neonatal cholestasis

Clinical Clues	Diagnosis
Onset of jaundice At birth: Few weeks after birth: Any point of time:	 GALD PFIC type II, galactosemia, tyrosinemia Mitochondrial Hepatopathy, HLH
Seizures	Hypoglycemia: Galactosemia, HFI Intracranial bleed: Cholestatic disorders (PFIC), advance liver failure with coagulopathy CNS infection: HSV
Pruritus	PFIC, Alagille's syndrome, Neonatal sclerosing cholangitis
Intrauterine growth restriction	GALD, MH
Maternal clues	Genital vesicles: HSV Oligohydramnios, megaplacenta: GALD Cholestasis of pregnancy or Pruritus on oral contraceptives: PFIC3 Acute fatty liver of pregnancy, hyperemesis: FAOD
Family history Consanguinity Repeated abortions or sib-loss Affected sib or sib death Gall stones	 Increased risk of autosomal recessive disorders GALD Galactosemia, tyrosinemia, MH, HLH, PFIC, Alagille PFIC-2 & 3

Clinical Clues	Diagnosis
Early-onset ascites	Galactosemia, tyrosinemia, GALD, MH, HLH, NPC
Delayed passage of meconium	Cystic Fibrosis
Presenting as acute liver failure	Galactosemia, tyrosinemia, GALD, MH, HLH, NPC, HSV, HFI
Findings on examination	
Scalp vesicles	HSV
Cataract	Galactosemia
Cherry red spot	NPC
Impaired vision	Septo-optic dysplasia
Hearing defects	PFIC1, Tight-junction protein (TJP2) mutations
Rickets (craniotabes)	Tyrosinemia
Hypotonia	MH, NPC, Zellweger's syndrome, Trisomy 21
Cardiac murmur	Alagille's syndrome
Facial Dysmorphism	Alagille's, Zellweger's syndrome, Trisomy 21
Skin rash	HLH, FAOD
Spleno-hepatomegaly	HLH, NPC
Shrunken liver	GALD
Hypoplastic (male) genitalia	Panhypopituitarism

GALD – Gestational Alloimmune Liver Disease, PFIC – Progressive Familial Intrahepatic Cholestasis, HLH – Hemophagocytic Lymphohistiocytosis, MH - Mitochondrial Hepatopathy, HFI – Hereditary Fructose Intolerance, HSV – Herpes Simplex Virus, FAOD – Fatty acid oxidation defect, NPC – Niemann Pick C

Table 3 – Clinical Features and diagnostic tests of common causes of cholestatic jaundice in infants associated with liver failure

	Clinical features	Supporting laboratory parameters	Diagnostic tests
Galactosemia	Feeding intolerance, vomiting, diarrhea, lethargy, developmental delay, hepatosplenomegaly, coagulopathy, hypoglycaemia, seizures, renal tubular dysfunction, cataract (bilateral), E.coli sepsis and rarely hemolysis.	Urine positive for non-glucose reducing substances (NGRS) while on lactose feeds	RBC Galactose-1 phosphatase uridyl transferase enzyme assay

	Clinical features	Supporting laboratory parameters	Diagnostic tests
Hereditary fructose intolerance	History of dietary exposure to fructose/sucrose, protracted vomiting, diarrhea, failure to thrive, hypoglycemia, seizures,	Positive urine NGRS, Fructose challenge test (obsolete nowadays), hypophosphatemia, hyperuricemia.	Pathogenic variants in ALDOB on genetic testing or deficient hepatic fructose 1-phosphate aldolase (aldolase B) activity on liver biopsy
Type 1 Tyrosinemia	Coagulopathy with or without cholestatic jaundice , hypoglycaemia, hepatomegaly, ascites	High alpha-fetoprotein (mean level: 160,000 µg/mL)	Increased urinary succinylacetone
Mitochondrial cytopathy	Onset in the first week of life or later, Multisystemic, neurological involvement in form of severe hypotonia, myoclonus or psychomotor retardation.	Plasma lactate >2.5 mmol/L, molar ratio of plasma lactate/pyruvate > 20:1,	Genetic mutational analysis for respiratory chain disorders and tandem mass spectrometry for fatty acid oxidation defects.
Gestational alloimmune liver disease	Presents usually few hours to days (sometimes weeks) after birth as hypoglycemia, coagulopathy, jaundice, anemia, ascites, anasarca, and splenomegaly with a shrunken liver.	High serum ferritin, low serum transferrin, high transferrin saturation (95 % to 100 %).	Lip or salivary gland biopsy shows iron deposition; MRI shows low signal intensity of pancreas and heart on T2 imaging.
Hemophagocytic Lymphohistiocytosis	Prolonged fever, hepatosplenomegaly, bleeding, skin rash, CNS abnormalities, jaundice.	Bicytopenia or pancytopenia, hyperferritinemia, hypofibrogenemia, hypertriglyceridemia	Molecular diagnosis or HLH-2004 (5 out of 8) diagnostic criteria
Niemann–Pick disease (type C)	Spleno – hepatomegaly, ascites, Hypotonia, ataxia, vertical supranuclear gaze palsy, developmental delay, seizures.	Positive filipin staining in cultured fibroblasts.	Molecular genetic testing of NPC1 and NPC2

	Clinical features	Supporting laboratory parameters	Diagnostic tests
Wolman's disease	Hepatosplenomegaly, ascites, vomiting, diarrhoea	High total serum cholesterol, low-density lipoprotein, and triglycerides; and low serum high-density lipoprotein, calcified adrenal glands	Deficient lysosomal acid lipase enzymatic activity in leucocytes (or fibroblasts)

E.coli - Escherichia coli, RBC – Red blood cell, CNS – Central nervous system, HLH – Hemophagocytic Lymphohistiocytosis, NPC – Niemann Pick C

Figures

Fig. 1 – Capsular and cut surface smooth with tiny nodules (<3 mm), cut surface is bile stained (gross photograph)

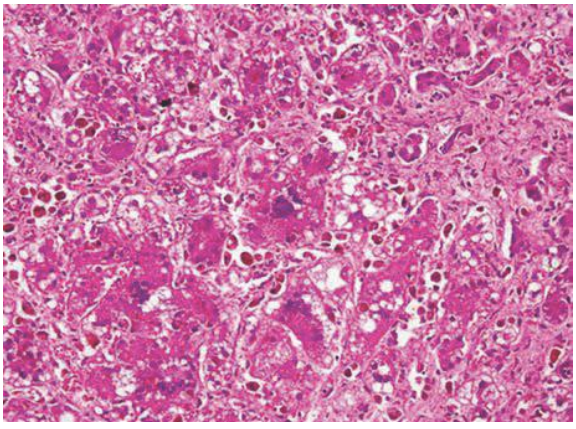

Fig. 2 – Hepatocytes show pseudo-acinar transformation, with micro- and macrovesicular steatosis and prominent giant cell transformation (H&E, 400×magnification)

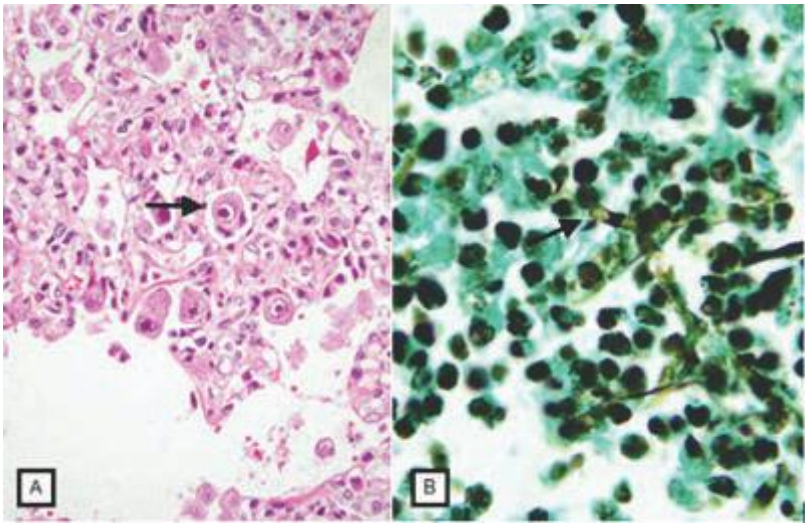

Fig. 3 – (A) CMV inclusions identified in pneumocytes lining the alveoli (H&E, 400×magnification) (B) Branching septate hyphae consistent with morphology of Aspergillus from the abscess in the left ventricular wall (D-Grocott stain 400×magnification)

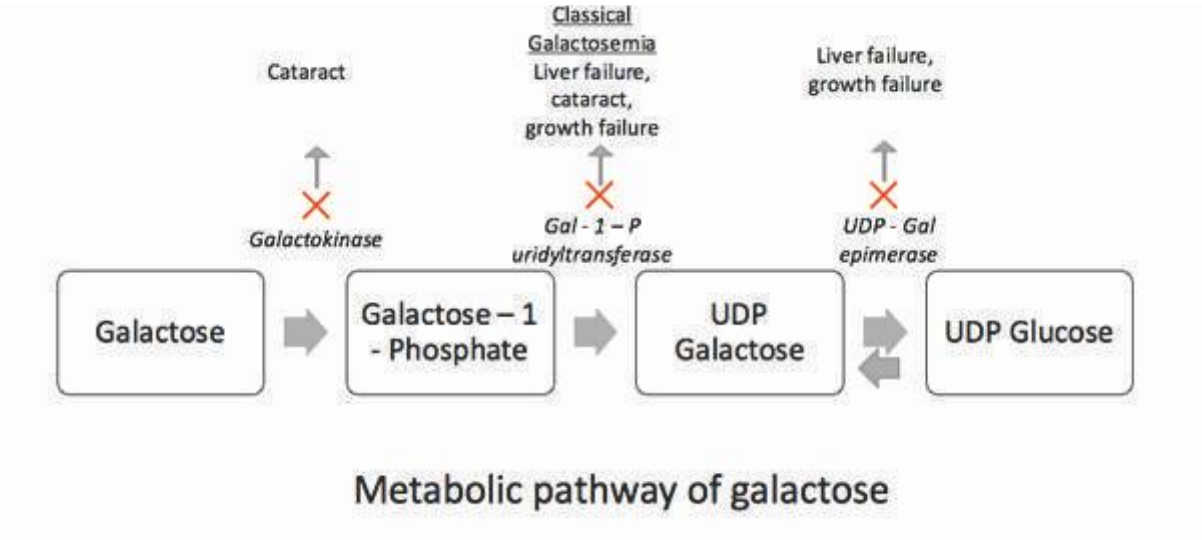

Fig. 4 – Galactose metabolism