Clinicopathological case

Idiopathic copper toxicosis in an infant

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Introduction
Copper is a trace element essential to certain biological processes of plants and animals. It serves as a co-factor of prosthetic groups in function of some enzymes and is responsible for the expression of specific genes.¹ Copper concentration in plants and animals is influenced by local conditions, particularly by the copper content in the earth and in the water.

Some foods such as viscera (liver, kidney) and some fruits and nuts have high copper contents.² Accumulation of copper in the liver has been observed in a variety of pediatric hepatic diseases that include Wilson’s disease,³ Indian childhood cirrhosis,⁴ and idiopathic copper toxicosis (ICT).⁵ The latter two have been related to copper-rich diets. ICT appears sporadically in the Indian continent with 15 cases per decade reported during recent decades.⁶ A clinical picture exists that is indistinguishable from it, which is called endemic tyrolean infantile cirrhosis.⁷ In Mexico, this clinical picture was reported by Valencia and Gamboa in 1993.⁸

In the absence of many cases, the existence of this entity should be kept in mind, and quantification of copper in blood and urine should be performed in any patient with cholestasis, anemia and malnutrition. However, it should be kept in mind that copper and ceruloplasmin concentrations may be normal or only slightly increased.

Clinical history summary
We present the case of a 1-year-old male whose evolution of his current condition began at 2 months of age with progressive and generalized icterus. The patient had insidious and nonprogressive abdominal distention at 2 1/2 months of age. There was also an increase in nonprogressive scrotal volume at the same time. Hypoacholia is denied.

Physical examination
During physical examination we found the following: weight 6085 g, height 58 cm, heart rate 160/min, respiratory rate 32/min, blood pressure 90/60 mm Hg, temperature 37.3ºC, capillary refill at 2 sec, head circumference 41 cm, and waist circumference 46 cm. His apparent age was younger than his chronological age. He had coarse, puffy facies, generalized jaundice and dry, brittle hair. There was bilateral palpebral edema, broad nasal bridge, low-set ears, chest restriction due to abdominal distention, and systolic murmur in the third left intercostal space (grade II/VI). Abdomen was globous due to ascites. Liver was 9 x 8 x 8 cm below the costal margin and spleen was 7 cm. There was a 2-cm reducible umbilical
hernia. Limbs were swollen. There was positive Godet sign and mild clubbing. Neurological exam was normal. Reflexes were OT ++/+++++ and petechiae of the sacrococcygeal region were observed.

Laboratory tests
Laboratory results were as follows: hemoglobin 10.6 g/dL, platelets 15,000/mm³, leukocytes 27600/mm³, sedimentation velocity 74%, lymphocytes 18%, monocytes 4%, bands 2%, glucose 64 mg/dL, calcium 7.5 mg/dL, phosphorus 2.5 mg dL, ammonia 105 mmol/L, prothrombin time 28 sec (T 12 sec), partial thromboplastin time 54.1 sec (T26 sec), INR 2.21, direct bilirubin 24.6 mg/dL, indirect bilirubin 3.92 mg/dL, total protein 6.2 g/dL, albumin 2.1 g dL, globulin 4.1 g dL, SGOT 383 IU, SGPT 384 IU, gamma glutamyl transferase 43 U/L, alkaline phosphatase 84 U/L, lactate dehydrogenase 406 U/L, urinalysis: pH 8.0, urinary density 1010, Hb 10 Eryth/mL, bile 6 mg/dL, urinary calcium 7.7 mg%, vol. 280 mL, gasometry: pH 7.41, PaO₂ 45 Pa CO₂ 32.9, HCO₃ 23.6, 28.9 CO₂T EB-0.4, SaO₂ 71.2, lactate 2.9.

The patient was seen by the Endocrinology Department and a diagnosis of hypothyroidism was made. The patient was started on levothyroxine (6.2 μg/kg/day). He was considered to be normal cardiologicaly. Abdominal ultrasound demonstrated free fluid in the abdominal cavity and bilateral nephrocalcinosis. Liver Doppler ultrasound was done. Portal vein demonstrated turbulent fluid, 10.3 cm/sec in main portion, 14.7 cm/sec of the right branch, and 21 cm/sec of the left branch. Right arterial hepatic resistance index was 0.85, capillary glucose 39 mg/dL. Endoscopic Tru-cut liver biopsy, bilateral inguinoplasty, umbilicoplasty and bilateral hydrocelectomy were carried out. Liver was dark green-purple in color with greenish nodular surface and surface venous network. Hepatic biopsy reported hepatic parenchyma with loss of architecture, numerous lymphocytes and proliferation of cholangioliths. Portal spaces were expanded with fibrosis and necroinflammatory bridges. No central veins were noted. Hepatocytes showed hydropic degeneration with clear cytoplasm, numerous Mallory bodies, and intra- and extrahepatic cholestasis with formation of biliary plugs in the cholangioliths. These were proliferated and accompanied by necroinflammatory bridges. Nuclei showed mild pleomorphism. Changes were compatible with ICT. The patient progressed to renal insufficiency, respiratory deterioration and died. He was hospitalized from March 21, 2008 to July 2008.

Radiological findings
Ultrasound showed hepatomegaly of homogeneous echogenicity. There was no dilatation of intra- or extrahepatic bile ducts (Figure 1). With the Doppler signal at the porta hepatis, the por-

![Figure 1. Abdominal ultrasound demonstrating homogeneous hepatic parenchyma without focal lesions, increase in the size of the liver and free perihepatic fluid.](image-url)
Idiopathic copper toxicosis in an infant

tal is observed with a speed of 10 cm/sec; at the splenic hilum it is 24 cm/sec. The hepatic artery had a resistance index of 0.85 (Figure 2).

On ultrasound, the kidneys are noted to be discretely increased in size. The pyramids, which are normally hypoechoic, are noted to be hyperechoic due to calcifications of poorly defined borders compatible with nephrocalcinosis (Figure 3). Suprahepatic veins were within normal limits. X-ray of chest and long bones show volume increase and soft tissue density.

Discussion

We presented the case of a 12-month old male. His medical history shows that there was moderate preterm low weight for gestational age according to the graphs adapted from Babson (32.4 weeks of gestation and weighing 1300 g, which is the second percentile). The child was the product of a vaginal birth. Spontaneous crying or respiration were absent, suggesting perinatal asphyxia. It is important to note that Cesarean delivery was necessary in order to avoid asphyxia and obstetric trauma. The infant also had sepsis and hyperbilirubinemia, apparently multifactorial, although no bilirubin levels or management were noted. He had intraventricular hemorrhage (IVH).

All of these events placed the infant at high neurologic risk; therefore, he was referred to this unit at 4 months of age. At 8 months of age a transfontanel ultrasound was obtained and showed cortical atrophy and ventriculomegaly, probably a sequelae of perinatal asphyxia and IVH. At 11-12 months he presented with central hypotonia. The findings are attributed to chronic bilirubinemic encephalopathy.

The question that remains is: why was he a preterm patient? It is mentioned in the medical history that there were apparently no maternal...
factors such as maternal infection or hypertension, but there is no detailed history of the pregnancy or laboratory tests. Among the notable factors that may trigger preterm labor are male gender, environmental factors including poverty, and limited resources for basic care and education, as discussed by the staff of the Department of Social Work.

At the age of 12 months when the patient was admitted for comprehensive study, the infant had severe malnutrition according to the anthropometric indicators indicated according to the Gomez classification, with a weight deficit of 58% expected for age. According to the description on admission (with edema of the extremities), it probably was a Kwashiorkor-type malnutrition and, according to the Waterlow classification, would be classified as an acute chronic malnutrition. The patient presented with severe psychomotor developmental delay. There are risk factors for this patient that would explain this, such as biological factors (birth weight <1500 g, gestational age ≤32 weeks, ventilatory assistance for >36 h, IVH) and established risk factors (perinatal asphyxia), as well as previously mentioned environmental factors and congenital hypothyroidism and a compatible phenotype with coarse facies, dry skin, wide anterior fontanelle and umbilical hernia.

Thyroid function tests support the clinical diagnosis and support the suspicion for secondary congenital hypothyroidism. The latter is a delayed diagnosis because at 6 months of age untreated hypothyroidism reduces IQ by 50%. It is important to mention that, in Mexico, in the majority of the cases, congenital hypothyroidism is primary with a frequency is 1:2537 cases (high in relation to other countries). Because at birth only <5% present with clinical data, it is recommended that neonatal screening be performed on all newborns. The patient presented with jaundice at 2 months of age with hyperbilirubinemia because of conjugated bilirubin. Diagnosis of cholestatic syndrome is added and also added to the syndromatic diagnoses is hepatosplenomegaly, documented clinically and imaging, as well as portal hypertension and chronic ascites.

Cholestatic syndrome
The causes of cholestatic syndrome are varied. It is mandatory to to rule out the following:

1. Infectious etiology-hepatocellular injury secondary to an infectious process is not excluded. The patient had negative serology for hepatitis A, B, EBV, VDRL; however, determination for cytomegalovirus, rubella, herpes, immunodeficiency virus and toxoplasmosis is missing.

2. Biliary duct obstruction—it is necessary in these cases to rule out biliary duct atresia. This problem presents itself in 1:8000 cases and consists of atresia or hypoplasia of any portion of the extrahepatic biliary system. Normally they are term children with adequate weight for gestational age who present with icterus at 2 to 3 weeks of age, accompanied by acholia. Abdominal ultrasound, which does not show the biliary vesicle, supports this possibility. However, this patient did not present with acholia and the liver biopsy was not compatible. A hepatic gammagram in order to observe excretion of the radioactive material into the intestine and intraoperative cholangiography would have been useful. It was not possible to totally exclude atresia of the biliary ducts. Alagille syndrome presents loss of the intrahepatic biliary conduits and is characterized by abnormal facies (wide forehead, hypertelorism, large straight nose with wide nasal ala and micrognathia), pulmonary stenosis-type cardiopathy or tetralogy of Fallot, anomalies of the vertebral column (butterfly vertebrae, hemivertebrae), ophthalmic alterations (embryotoxic), growth delay and mental retardation. This possibility is ruled out because none of these findings were made.
3. Congenital diseases of metabolism—humans are known to have >2000 distinct enzymes so there should exist <2000 congenital diseases of metabolism, although only ~400 are known. Expanded metabolic screening is useful in these cases (we lack this study at least in the clinical history). I will mention those compatible with this clinical picture:

a) Tyrosinemia is a progressive multisystemic disorder that affects the liver, kidney and peripheral nervous system. Progressive liver dysfunction begins in childhood and in advanced ages. A characteristic clinical picture is seen but, in some patients, the disease is rapidly progressive and patients develop liver failure at 1 year of age. A variable degree of renal dysfunction is also characteristic (renal tubular acidosis, glomerulosclerosis and renal failure) and the presence of nephrocalcinosis in 33% of cases. The patient, despite having liver involvement, did not present with renal tubular acidosis and renal function was normal at admission. The diagnosis is supported by tyrosine levels and remains as a remote possibility.

b) Sphingolipidoses (including Gaucher-Colman and Niemann-Pick disease). These present with hepatic fibrosis. What is also striking is the increased cholesterol and TGs, which is not correlated with our patient. Biopsy revealed intralysosomal lipids, so this was ruled out.

c) Type 1 glycogenesis. Hepatomegaly with lactic acidosis and hypoglycemia are present but not confirmed on admission. The patient presents with hyperlactatemia and hypoglycemia during the course of severe disease, which correlates more with the state of hemodynamic deterioration and the development of liver failure present in the final stage of the disease.

d) Wilson’s disease. An autosomal recessive disorder of copper metabolism that is reported to be rare in patients <5 years of age. Dr. Fontana of the University of Michigan recently emphasized that this possibility should always be considered in cases of hepatosplenomegaly accompanied by hepatic insufficiency of catastrophic evolution and whose cause is not well established. There are other sites of accumulation of copper toxicity in Wilson’s disease such as central nervous system, eyes and kidneys.

In our patient, pituitary dysfunction may have been due to the accumulation of copper. Nephrocalcinosis is a finding described in this disease. It would have been helpful to have had an MRI of the brain. On physical examination Kayser-Fleisher rings were not described, but these may be absent in 50% of cases.

Diagnosis is made with the determination of low levels of serum ceruloplasmin and high levels of copper in blood and in urine. Liver biopsy with special stains can detect copper deposits at the cellular level and quantify the concentration of copper per gram of liver tissue. I cannot rule out this disease because I did not have the laboratory studies.

e) Iron-hoarding disease. This refers to neonatal hemochromatosis where iron is deposited in the liver, pancreas, skin, and endocrine system (thyroid). In this disease there is a history of prematurity, intrauterine growth retardation, and a clinical picture of hepatitis with hepatomegaly and cholestatic syndrome. It can occur during the first weeks of life and, in most cases, develops as a fulminant hepatitis. Diagnosis is confirmed with elevated plasma iron, decreased transferrin, elevated transferrin saturation, and elevated ferritin. Liver
biopsy is also helpful in the diagnosis because special stains can detect iron deposits as well as the quantification of iron per gram of liver tissue. This cause is not ruled out.

4. Among the miscellaneous causes are hepatotoxicity by xenobiotics: by medications (paracetamol), metals (acute ingestion of copper sulfate, iron), fungi of the amanita species (liver necrosis) and herbal (derived pyrrolizidine producing veno-occlusive disease, mullein) as well as cystic fibrosis and prolonged parenteral feeding.

Other diagnoses
Other diagnoses included hepatic insufficiency. The patient had hypoalbuminemia, coagulation disorder with INR >1.5 with increased bilirubin and hypoglycemia.

Infectious processes associated with nosocomial pneumonia
Evolution
Liver biopsy was performed due to deterioration of the patient’s clinical status. The patient presented with nosocomial infection, metabolic and electrolyte disturbances, signs of acute lung injury and shock with multiorgan failure. Of note is the steady decline of hemoglobin despite transfusions which, coupled with coagulation disorders, required suspicion of blood loss or hemolysis.

Final diagnoses
Our patient was a 1-year old male infant with acute or chronic malnutrition, psychomotor delay, secondary congenital hypothyroidism, status postbiopsy, status posthernioplasty-hydrocele, hepatosplenomegaly, portal hypertension, cholestatic syndrome, nosocomial pneumonia, hepatic insufficiency and status postarrest.

Cause of death
Multiple organ failure and mixed shock.

Description of histopathological findings
Liver biopsy showed hepatocytes with cholestasis, pseudoacinar transformation, and clear cytoplasm where there are numerous Mallory bodies and interstitial fibrosis. In the ultrastructure study, mitochondria of the hepatocytes did not show morphological changes (Figure 4). According to these findings, a diagnosis compatible with ICT was made.

At autopsy, the patient had generalized jaundice, abdominal distension, collateral venous network, umbilical hernia, bilateral hydrocele and eyes with dark coloration seen in the limbus corresponding to Kayser-Fleischer rings (Figure 5). In children, Kayser-Fleischer rings have been described in various diseases that present with cholestasis (Table 1) and are due to copper deposition in the Descemet membrane.

The liver was increased in size and weight, and its outer surface and cut was micronodular, green in color and with nodules measuring <3 mm. Histological examination showed regenerative nodules surrounded by connective tissue septa with proliferation of cholangioles. There is interstitial fibrosis within the nodules. Hepatocytes shows numerous Mallory bodies, cholestasis, and degeneration of hepatocytes with polymorphonuclear satellitosis.

In the immunohistochemical reactions, Mallory bodies were positive for cytokeratins of low-and high-molecular weight and staining intensity was greater for high-molecular-weight cytokeratin (Figure 6). There was no histochemical staining to demonstrate copper; therefore, orcein staining was performed to demonstrate the deposition of copper-associated protein, which was positive in the hepatocyte cytoplasm.

Kidneys were enlarged and showed evidence of acute tubular necrosis and casts of bile pig-
Idiopathic copper toxicosis in an infant

Figure 4. (A) Hepatocytes with numerous Mallory bodies (arrow) and cholestasis. (B) Microphotography of the nodules of regeneration with interstitial fibrosis (Masson trichromic). (C) Mitochondria do not show ultrastructural alterations (arrows).

Figure 5. (A) Conjunctival icterus and Kayser-Fleischer rings were observed postmortem. (B) Abdomen is distended with collateral venous network and umbilical hernia.

The brain showed subcortical atrophy and dilatation of the ventricles. Different histological sections of the cortex and basal ganglia demonstrated

ments. Microscopic examination of the digestive tract showed only changes of hypoxic-ischemic visceral myopathy secondary to shock. Lungs demonstrated resolving acute pneumonia. In the bone marrow, precursors of the three series showed no alterations; there was mild osteopenia. The thymus was small and atrophic.

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Table 1. Diseases that present with cholestasis in those patients with Kayser-Fleischer rings

- Wilson’s disease
- Chronic hepatitis
- Primary biliary cirrhosis 3
- Neonatal cholestasis 4
- Idiopathic copper toxicosis
- Cryptogenic cirrhosis

chronic changes characterized by neuronal loss and metabolic encephalopathy, as well as recent multifocal necrosis of neurons (Figure 7).

Congenital malformations found in this patient were *cavum septum pellucidum*, foramen ovale and atrioseptal defect of 2 mm, and mesocolon capillary hemangioma of the cecum (Figure 8). These latter were not detected clinically.

Metabolic diseases with a morphological pattern of liver cirrhosis include Wilson’s disease and ICT, among others (Table 3). Morphological changes may be indistinguishable during the initial stages, but there are some differences that make their separation possible when biopsies are analyzed. The most important data are age at symptom onset, family history or history of exposure to copper utensils. Molecular study is also recommended.

The experience of the Hospital Infantil de Mexico Federico Gomez in the case of cirrhosis associated with copper toxicity was reported by Valencia Gamboa. The four patients were males and three were <2 years of age, as the patient presented in this report.

**Figure 6.** (A) Liver on sectioning with green color and micronodular surface. (B) Mallory bodies are positive for high-molecular-weight cytokeratin (immunohistochemical reaction). (C) The majority of the hepatocytes have Mallory bodies and bile. (D) Deposits of copper-associated protein in hepatocytes (arrow) (orcein stain).

**Figure 7.** (A) Brain on sectioning with dilation of ventricles and *cavum septum pellucidum*. (B) Recent neuronal necrosis in the cortex is observed.
Idiopathic copper toxicosis in an infant

Figure 8. (A) Solid ovoid tumor attached to the cecum in the mesenteric border. (B) The tumor is composed of thin-walled blood vessels (hematoxylin/eosin, x400).

Tabla 2. Diseases that present with a morphological pattern of hepatic cirrhosis

<table>
<thead>
<tr>
<th>Liver diseases associated with copper</th>
<th>Idiopathic copper toxicosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilson’s disease</td>
<td>Idiopathic copper toxicosis</td>
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<tr>
<td>• Steatosis</td>
<td>• Minimal steatosis</td>
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<tr>
<td>• Chronic hepatitis</td>
<td>• Globoid degeneration</td>
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<td>• Mallory bodies +</td>
<td>• Mallory bodies +</td>
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<td>• Cholestasis ±</td>
<td>• Cholestasis + to +++</td>
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<td>• Macronodular cirrhosis</td>
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<td>&gt;5 years</td>
<td>&lt;2 years</td>
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<tr>
<td>• Exposure to Cu++ ±</td>
<td>• Exposure to Cu++</td>
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<tr>
<td>• Autosomal-recessive inheritance</td>
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<tr>
<td>• ATP7B</td>
<td>• Autosomal-recessive?</td>
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Tabla 3. Cases of cirrhosis associated with copper toxicity

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<thead>
<tr>
<th>Metabolic diseases with a pattern of cirrhosis</th>
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</thead>
<tbody>
<tr>
<td>• Hereditary tyrosinemia</td>
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<tr>
<td>• Galactosemia</td>
</tr>
<tr>
<td>• Glycogenesis type IV</td>
</tr>
<tr>
<td>• Indian childhood cirrhosis</td>
</tr>
<tr>
<td>• Idiopathic copper toxicosis</td>
</tr>
<tr>
<td>• Neonatal hemochromatosis</td>
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<tr>
<td>• α-1 antitrypsin deficiency</td>
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<td>• Wolman’s disease</td>
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Final diagnoses are as follows:

- Primary disease was ICT
- Concomitant disorders included micronodular liver cirrhosis, severe cholestasis, hepatomegaly 300 g vs. 280 g, bilirubin nephropathy, portal hypertension, resolving acute pneumonia, thymic atrophy, cerebral atrophy with dilated cerebral ventricles, metabolic encephalopathy, cerebral edema 900 g vs. 852 g, acute hypoxic encephalopathy.
- Independent alterations were hypothyroidism, umbilical hernia, status post bilateral inguinalplasty, mesocolon capillary hemangioma, 2-mm septal foramen ovale, and cavum septum pellucidum.

Age of symptom onset of ICT reported in the literature varies from 2 months to 10 years, as was the case with our patient. Three groups of onset can be distinguished: infantile-1) before or close to age 2 years, 2) late start-about 5 years of age, and exceptional delay-about 10 years of age.10-12

Indian childhood cirrhosis occurs between 6 and 18 months of age13 and symptoms of Wilson’s disease begin at ~6 years of age.14 In most cases, ICT is characterized as a disease of insidious onset, rapid progression and death in the first year, as was the case with our patient. Occasionally it has a chronic presentation that lasts for years and results as hepatic insufficiency.

Laboratory exams that allow us to make the diagnosis are high concentrations of copper in blood and liver (between 190 and 360 mg/g of dry liver weight), the normal concentration is ≤50 µg.8,10,11,14 An increase in copper excretion in the urine has also been observed. Ceruloplasmin is usually normal or slightly elevated. The study that confirms this diagnosis is not only a liver biopsy, but the amount of copper that is stored in the liver.
The main etiologic factor of this disease is the constant use of utensils made of copper, because copper contamination of food can occur, such as is the case with milk, and can produce accumulation of copper and produce the disease. In Mexico there are regions such as the town of Santa Clara del Cobre in the state of Michoacan where cooking utensils are made of this material. Even though it appears that this is not a very common disease, it must be kept in mind because it can be treated early with D-penicillamine and liver transplantation can be performed.

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References