Aims

- Review clinical presentation, causes and genetic defects leading to oxalate stones
- Understand importance of early recognition of primary hyperoxaluria to minimize systemic oxalosis and improve patient outcome
- Discuss the principles of medical therapy, dialysis, transplant and post-transplant care for primary hyperoxaluria
Overview: Frequency of Pediatric Kidney Stones

- **USA**
  - Infrequent (no natl report)
  - More often in Caucasians, rare in African Americans
  - Male:female ~3:2
  - Over 50% identifiable metabolic origin
  - Endemic stones very rare

- **Europe**
  - 1-2 children/million population
  - Upper tract more common
  - UK: ~90% infection-related

- **Developing Countries (Middle East, Southeast Asia, India, Eastern Europe)**
  - More frequent
  - Male:female 5:1
  - Endemic stones common
    - Younger age, rural setting

- **Transitional Countries (Armenia, Pakistan, Thailand, Turkey)**
  - Decrease in endemic stones
  - Male:female 3-4:1
  - More identifiable metabolic disorders
Overview: Risk factors for Pediatric Kidney Stones

- **Infection**
  - Urea-splitting bacteria
  - Congenital UT anomalies
  - Urinary stasis

- **Genetics**
  - Congenital UT anomalies
  - Common
    - Hypercalciuria
    - ? hyperuricosuria
  - Rare disorders
    - Cystinuria
    - Purine metabolism disorders
    - Cystic fibrosis, Wilson’s
    - **Hyperoxaluria/Oxalosis**

- **Diet**
  - High oxalate, calcium
  - High grain, low prot (malnutrition)
  - High animal prot, gluc/sucrose
  - Ketogenic
  - Low fluid intake; dehydration

- **Surgical**
  - Bladder augmentation, Mitrofanoff channels, etc
Urine Oxalate Crystals

Not seen in urinalysis in primary hyperoxaluria
Secondary (Acquired) Hyperoxaluria

- **Enteric hyperoxaluria: chronic diarrhea**
  - Diarrhea → less intestinal Ca for oxalate binding
  - Increased intestinal oxalate absorption
  - May occur in inflammatory bowel disease, steatorrhea, pancreatic insufficiency, biliary cirrhosis, short-bowel syndrome

- **Bariatric surgery**
  - Jejuno-ileal bypass for obesity → malabsorption
  - Kidney stones with risk for renal failure
  - Urinary oxalate 85 mg/day (normal <40) in 132 pts with stones
  (Asplin & Coe, J Urol 2007;177:565)

- **Vitamin C excess** (>1 g daily) → conversion to oxalate; avoid in CKD

- **Hydroxyproline ingestion**
  - Amino acid of collagen; found in gelatin and meat
  - Dietary hydroxyproline normally accounts for only 5-20% of urine oxalate excretion
  - Animal model for oxalosis (PH-1 & PH-2): gelatin >5g/day in diet → very increased urinary oxalate and glycolate
Primary Hyperoxaluria

- **Type I (PH-1):** Most common 72%*
  - Enzyme deficiency/organelle mistargeting
    - alanine:glyoxalate aminotransferase (AGT)
    - In liver
- **Type II (PH-2):** 10%*
  - Enzyme deficiency
    - glyoxalate reductase/hydroxypyruvate reductase (GRHPR)
    - In many organs, but highest in liver
- **Type III (PH-3):** 9%*
  - Enzyme deficiency
    - 4-hydroxy-2-oxoglutarate aldolase (HOGA)
    - In liver and kidney
- **Non PH-1,2,3:** 8%*; Unknown 1%*

*% from Rare Kidney Stone Consortium Registry as of July 2015
Primary Hyperoxaluria

- Rare in frequency
  - 1:120,000 live births in France
  - 1-2% of end-stage renal disease USA, Europe, Japan, but 13% in Tunisia (consanguinity)

- Autosomal recessive disorders
  - Monogenic = mutation in one gene
  - Phenotype variable: multiple mutations
  - Compound heterozygotes with disease

- Defects in oxalate metabolism → toxicity
  - from overproduction of oxalate
    - Kidney—nephrolithiasis, nephrocalcinosis
    - Other tissue deposition/systemic oxalosis
      - bone, bone marrow, eye (retina), heart, peripheral blood vessels, joints, skin
Primary Hyperoxaluria

X PH-1

X PH-2

X PH-3

↑↑↑ OXALATE (blood, urine)

Systemic oxalosis: Retina, heart, bone, bone marrow, skin, joints, blood vessels

Kidney stones

Nephrocalcinosis

Cochat & Rumsby, NEJM 2013;369:650
Primary Hyperoxaluria: Clinical Presentation

- 80% symptomatic prior to age 20yo, 15% <1yo
- **Presenting symptoms**
  - Kidney stone, renal colic—most common
  - Hematuria; dysuria, urinary tract infection (UTI)
  - Renal failure
    - Severe infantile form: renal failure/ESRD <1 yo
    - Others: ESRD by median age 15-25 yo (depending on series)
    - In 30-50% of children, ESRD present at time of diagnosis
    - 10% diagnosed with PH-1 following renal transplant
- **Imaging** by renal ultrasound (RUS): multiple stones, nephrocalcinosis
- **Screening** after sibling diagnosed (24 hr urine, RUS)
- **Late manifestations from systemic oxalosis**
  - Occurs months to years after reach CKD stage 4
  - Other organ deposits in bone, retina, bone marrow, myocardium, peripheral blood vessels, joints, skin
Primary Hyperoxaluria, Type 1
Nephrolithiasis, Nephrocalcinosis, ESRD

Presentation with ESRD in 6 month old
Primary Hyperoxaluria Type 1

Presentation with stones at age 5 years

ESRD by age 7 years
Primary Hyperoxaluria (PH): Stones with Peculiar Structure

- >95% Ca oxalate monohydrate (whewellite) stones in PH
- PH-1 & PH-3 stones:
  - Usually white or pale yellow compared to dark brown surface of idiopathic calcium oxalate stones
  - Irregular aggregates of crystals with extreme porosity and no clear cut growth structure
  - Scanning EM: inhomogenous, loose, irregular crystal aggregates & spheres suggesting rapid formation of stone
Primary Hyperoxaluria: Gross Pathology

Nephrectomy specimen

- stones
- medullary
cortical
- nephrocalcinosis
- nephrocalcinosis
Primary Hyperoxaluria Histology: oxalate deposition in cortex by LM
Primary Hyperoxaluria: Non-renal oxalate deposition

Occurs late in disease (years after CKD stage 4)

- Eye
  - Crystalline retinopathy
  - Blindness from reactive choroid neovascularization

MayoMedicalLaboratories.com/communique
2013;38(No.4):1-9
Primary Hyperoxaluria:
Non-renal oxalate deposition

Occurs late in disease (years after CKD stage 4)

- **Cardiovascular system**
  - Heart block from deposition oxalate crystals in cardiac conduction system
  - Peripheral vascular disease: arterial occlusion/spasm, livedo reticularis, acrocyanosis, Raynaud phenomenon of hand, intermittent claudication, gangrene
Primary Hyperoxaluria: Non-renal oxalate deposition

Occurs late in disease (years after CKD stage 4)

- **Bone**: pathologic fractures, pseudo fractures, bone-in-bone appearance of oxalate deposition
- **Bone Marrow**: pancytopenia, anemia, resistance to ESA
- **Joints**: Soft tissue calcifications; limited movement
- **Skin**: Extrusion oxalate from nails & under skin
Primary Hyperoxaluria: Genetics
Primary Hyperoxaluria

- **Type I (PH-1)** = most common
  - **AGXT** gene on chromosome 2q37.3
  - Over 170 mutations in all 11 exons (2014)
  - Encodes for 86 kDa protein enzyme named alanine:glyoxalate aminotransferase (AGT)
Primary Hyperoxaluria, PH-1

- Mutation in AGXT gene
- Alanine:glyoxylate aminotransferase (AGT) deficiency/absence in hepatocyte peroxisomes
Primary Hyperoxaluria, PH-1

Idealized Hepatocyte
Primary Hyperoxaluria, PH-1

- Serine
- Pyruvate
- Glycine
- Glyoxylate
- Alanine
- Glyoxalate
- Hydroxypyruvate
- Oxalate
- Glycolate
- NAD(P)H
- NAD(P)
- LDH
- GR
- AGT

Peroxisome

Cytosol

Hepatocyte

Pyridoxine (cofactor)
Primary Hyperoxaluria, PH-1

**Peroxisome**
- serine
- pyruvate
- alanine
- glycine
- glyoxylate
- hydroxypyruvate

**Cytosol**
- glycine
- oxalate
- LDH (catalyzes conversion of glyoxylic acid to glycolic acid)
- GR (glutathione reductase)
- NAD(P)

**Peroxisome**
- AGT (gallamine)
- NAD(P)H

**Hepatocyte**
- Pyridoxine (cofactor)
- GO (glycolate oxidase)

**Oxalate**
- X
- PH-1
- X

**Glucogenic Oxidation**
- glycolate
- glyoxalate
- oxalate
Primary Hyperoxaluria, PH-1: Mistargeted AGT variant in mitochondrion

Idealized Hepatocyte
Primary Hyperoxaluria

- **Type II (PH-2)**
  - *GRHPR* gene on chromosome 9p13.2
  - At least 30 mutations (2013)
  - Encodes for glyoxalate reductase/hydroxypyruvate reductase (GRHPR)
    - Location in many organs, but highest in liver
    - Deficiency GRHPR cytosol, not peroxisome
Primary Hyperoxaluria, PH-2

![Diagram of metabolic pathways involving oxalate, glycine, pyruvate, alanine, and other metabolites in peroxisomes, cytosol, and hepatocytes/proximal tubules.]

- Oxalate is generated from hydroxypyruvate in the peroxisome.
- Glycine is converted to glyoxylate and pyruvate by AGTs.
- Alanine is transformed into pyruvate and glycine.
- D-glycerate is produced from hydroxypyruvate in the cytosol.
- L-glycerate is synthesized from D-glycerate.
- Glycolate is converted to oxalate in the hepatocyte/proximal tubule.
- NAD(P)H and NAD(P) are involved in redox reactions.
- LDH and GR enzymes catalyze reactions involving glycerates and oxalate.

Gluconeogenesis occurs in hepatocytes and proximal tubules.
Primary Hyperoxaluria, PH-2

- Serine $\rightleftharpoons$ pyruvate $\rightleftharpoons$ glycine $\rightleftharpoons$ glycine

- Alanine $\rightleftharpoons$ glyoxylate

- Glyoxylate $\rightleftharpoons$ pyridoxine

- Hydroxypyruvate $\rightarrow$ hydroxypyruvate

- L-Glycerate $\rightarrow$ L-Glycerate

- NAD(P)H $\rightarrow$ NAD(P)

- D-glycerate $\rightarrow$ D-glycerate

- Oxalate $\rightarrow$ oxalate

- Glycolate $\rightarrow$ glycolate

- Oxalate, X

- PH-2, X

- Hepatocyte, proximal tubule
Primary Hyperoxaluria

- **Type III (PH-3), ~10% PH, but no ESRD**
- **HOGA1** gene on chromosome 10q24.2
  - 19 mutations (2013)

- Encodes for 4-hydroxy-2-oxoglutarate aldolase (HOGA) in liver and kidney (mitochondria)
  - Catalyzes synthesis of mitochondrial glyoxylate from 4-hydroxy-2 oxoglutarate in the pathway of hydroxyproline metabolism
  - Deficiency results in excess production of oxalate and kidney stones; 50% present as child >5yo
  - No ESRD yet described
Primary Hyperoxaluria
Diagnosis: screening

- **24 hour urine oxalate** greater than ~2x upper limit of normal (if normal GFR)
  - >0.7 mmol (63 mg)/1.73 m2 per 24 hr; usually >1 in primary hyperoxaluria
  - Only after age 2 yrs old, when GFR matures to adult standard 100-120 ml/min/1.73m2
  - May be lower, if GFR <30 ml/min/1.73m

- **Plasma oxalate**, if CKD >Stage 3b (G FR <45)
  - CKD, Stages 1-3: may be normal, <1.8 mc mol/L
  - CKD, Stages 4-5: usually >20 mc mol/L
  - Dialysis: >80 mc mol/L in PH-1 vs. <60 in other dialysis patients
Primary Hyperoxaluria
Diagnosis: Urine screening

24 hour urine (best) or Random urine with creatinine (Cr)

<table>
<thead>
<tr>
<th>Urine</th>
<th>PH-1</th>
<th>PH-2</th>
<th>PH-3</th>
<th>Secondary Hyperoxaluria</th>
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<td>oxalate</td>
<td>↑↑↑↑</td>
<td>↑↑↑↑</td>
<td>↑↑↑↑</td>
<td>↑↑↑</td>
</tr>
<tr>
<td>glycolate</td>
<td>↑/Normal</td>
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<td>Normal/↑</td>
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<tr>
<td>glycerate</td>
<td>Normal</td>
<td>↑</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>4-hydroxy-2-oxoglutarate (HOG)</td>
<td>Normal</td>
<td>Normal</td>
<td>↑</td>
<td>Normal</td>
</tr>
<tr>
<td>calcium</td>
<td>↓/low</td>
<td>↓/low</td>
<td>↑/↑↑↑</td>
<td>Normal/low</td>
</tr>
</tbody>
</table>
Primary Hyperoxaluria: Diagnosis

- Genetic screening (blood test)
  - Confirm diagnosis + test for carriers in families with known history PH
  - AXG T full gene analysis for PH-1
    - 3 common mutations in 70% PH-1: c.33dupC, p.Gly170Arg (c.508G>A), and p.Ile244Thr (c.731T>C)
  - GRHPR full gene analysis for PH-2
    - 2 common mutations in 30% Caucasian, 15% Asian: c.103delG and c.403_404+delAAGT
  - HOGA1 gene analysis for PH-3: research only

- If negative, need liver biopsy for enzyme activity testing
Primary Hyperoxaluria: Diagnosis

- Liver biopsy for enzyme activity
  - Definitive testing for enzyme deficiency
    - Deficiency immunoreactive AGT protein and enzyme activity (42% of PH-1)
    - Presence of immunoreactive AGT protein, but deficient enzyme activity (16% of PH-1)
    - Presence of both, but site of action mitochondria, not peroxisomes (mistargeting PH-1)
    - Deficiency of GRHPR activity (PH-2)
  - No test for HOGA enzyme activity (PH-3)
Primary Hyperoxaluria: Pre-natal Diagnosis

- Fetal liver biopsy with ultrasound guidance for enzyme activity (1988)
  - Only in 2nd trimester
- Chorionic villus biopsy for DNA mutation analysis (1996)
  - 1st trimester
Primary Hyperoxaluria: Diagnosis

- Early diagnosis may lead to better long term outcome
  - Aggressive therapy to prevent oxalate accumulation in body (systemic oxalosis)
  - If pyridoxine responsive, early pyridoxine may retard onset of kidney failure
- High index of suspicion for PH in children with multiple Ca-oxalate stones or onset in early childhood
Primary Hyperoxaluria: Treatment-Symptomatic

- **High fluid intake** (2000-3000 mL/m²/day)
  - Gastric tube may be necessary to maintain intake, especially during illness and at night

- **Increase urine pH for ↑solubility Ca-oxalate**
  - Urine pH 6.2-6.8 with K citrate; Na citrate if CKD
  - Na/K phosphate supplement; 20-30mg/kg/d of P

- **Thiazides to decrease urine calcium (esp pH-3)**
  - AVOID loop diuretics (furosemide); increase urine Ca

- **Avoid significant intake Vitamin C or D**

- **Remove obstructing stones**
  - Ureteroscopy, percutaneous, or lithotripsy
Primary Hyperoxaluria: Treatment-Medicine

- **High dose pyridoxine (vitamin B6) in PH-1**
  - Cofactor of AGT enzyme: promotes conversion of glyoxalate to glycine, not oxalate, when partial deficiency of AGT
    - Very effective for homozygous AGXT mutation p.Gly170Arg
    - Effective in some symptomatic heterozygotes
    - Not effective in most severe forms PH-1, but trial should be given to all PH-1 patients
  - Starting dose: 2-5 mg/kg/day, then increase to 10-20 mg/kg/day
  - Sensory neuropathy at very high doses
Primary Hyperoxaluria: Treatment-Other

- **Oxalobacter formigenes**
  (Hoppe et al, Nephrol Dial Transplant, 2011;26:3609-3615)
  - Colonic degradation of endogenous oxalate
    - Oxalobacter uses oxalate as sole energy source
    - Oxalobacter non-toxic; eliminated in feces
    - Two 4 wk pilot studies showed effectiveness (13 PH pts; nl eCr)
  - 42 pts received study medicine BID for 24 wks
    - 19 pts: Oxalobacter enteric coated caps (dose $10^7$ cfu Oxalobacter formigenes)
    - 23 pts: Placebo caps
  - Safe, but not effective
    - Oxalobacter vs. placebo: 20% vs. 10% ↓urine oxalate/Cr
    - Well tolerated; no serious adverse events
Primary Hyperoxaluria: Treatment-ESRD: Dialysis

- **Avoid long term dialysis**—urgent to proceed with liver-kidney transplant

- **Conventional 3X week HD or PD not effective** to overcome excess oxalate production
  - Oxalate generation in PH-1: 4-7 mmol/1.73m²/day
  - HD removal: Adult 1-2, child 3-4 mmol/1.73m²/day
  - PD removal about 1/3 HD removal

- **Goal: Minimize systemic oxalosis**
  - Reduce and maintain plasma oxalate <30-45 mc mol/L = supersaturation threshold for tissue deposition of oxalate
Primary Hyperoxaluria: Treatment – Dialysis

- Daily (6-7d/week) high efficiency hemodialysis
  - High blood flow, high flux, large surface area dialyzer
  - Pre-dialysis plasma oxalate often 100-200 mmol/L
  - ~60-80% removal plasma oxalate post-HD, then back to 80% of the pre-dialysis level by 24 hrs
- Nocturnal hemodialysis for increased HD time
- Combined HD & PD useful in some infants
  - PD removes about 1/3 amt HD; together PD+HD more effective, especially in infants
    - Kansas City: Catherine Knight, Poster at ADC 2008
Primary Hyperoxaluria: Treatment-Transplant

- Since 1976, over 200 pts treated with transplant
  - Staged kidney, then liver TX—not effective for PH-1
    - High incidence of renal loss; EDTA ~20% 3-yr graft survival
  - Kidney transplant alone done for PH-2 (limited f/u)
- Combined liver-kidney TX preferred for PH-1
  - 2000-2009, n=24 1st transplants: 3-year graft survival 84%
- Preemptive liver transplant (Kemper, Urol Res, 2005;33:376)
  - Rationale: cure metabolic defect before kidney badly damaged
  - Reviewed 11 patients 1-38 yo, GFR 15-98/1.73m² pre-TX
  - Follow-up: 2-13 yrs; 1 death@1 yr, 1 re-TX
  - Outcome: 2 kidney TX 5-6yrs; 8 with GFR 37-93
Primary Hyperoxaluria: Transplant Outcome

- **Best outcomes with**
  - Simultaneous liver-kidney TX
  - Pre-emptive, if possible
  - No special immunosuppression protocols
  - **AVOID** delayed graft function post-TX
  - If poor urine output, do high flux CRRT or daily high efficiency HD post-TX
- Post-transplant hyperoxaluria protocol
  - High fluid intake around the clock
  - Meds to promote urine Ca-oxalate solubility
  - **AVOID** loop diuretics (furosemide)
Primary Hyperoxaluria: Treatment-Post-TX Surgery

- Post-transplant hyperoxaluria therapy
  - High fluid intake 2-3 L/m2/day, including overnight
    - Gastric tube, NG tube for overnight fluids
    - Rapid response to vomiting/diarrhea to prevent dehydration
  - Oxalate crystal inhibitors to promote urine excretion
    - Na/K citrate, Na/K phosphate
  - Duration of therapy depends on systemic body burden of oxalate
    - 6 mon to 5 years
Primary Hyperoxaluria: Post-TX Monitoring

- Transplant kidney at risk for oxalate damage, stones even after combined liver-kidney transplant
  - Oxalate excreted only in urine
  - Body burden of oxalate must be excreted in urine after TX
    - Often takes years post-TX
- Plasma oxalate level
  - Monitor monthly first 6 months, then quarterly
  - May be normal well before 24h urine oxalate normal
- Urine oxalate excretion
  - Random urine oxalate: goal <0.3 nmol/L (Mayo Lab)
  - 24hr urine oxalate monthly x3, then quarterly
- Ophthalmology exam every 6 months until clear
- Bone x-rays every 6 months until healed
Primary Hyperoxaluria: Future Treatment

- Restoration of defective enzyme
  - Chemical chaperone
    - Small molecules that increase protein stability and normalize folding, oligomerization and targeting pathways for missense mutations
    - Examples: pyridoxal 5’-phosphate, amino oxycetic acid
  - Hepatocyte cell transplant
  - Recombinant gene therapy/enzyme replacement
    - Adeno-associated virus (AAV) vector in mouse study
    - Difficulty: must target majority of hepatocytes for gene
Primary Hyperoxaluria: Summary-1

- **Primary hyperoxaluria (PH)** is a rare inborn error of metabolism that **leads to kidney damage through production of excess oxalate** and formation of multiple oxalate **kidney stones and nephrocalcinosis**

- Depending on the severity of the genetic enzyme deficiency, patients **may reach ESRD in infancy up to early adulthood**

- **Definitive diagnosis** by blood test for full gene analysis, but if neg, may require liver biopsy for enzyme testing in PH-1 and PH-2
Primary Hyperoxaluria: Summary-2

- Medical treatment with high fluid intake, high dose pyridoxine, and oxalate crystal inhibitors may retard progression to ESRD
- Timely liver-kidney transplant is the preferred modality for ESRD in PH-1, with daily hemodialysis (±PD) required for best outcome while waiting for TX
- Post-TX, medical therapy is necessary for up to several years to prevent damage to the new kidney until the body burden of oxalate can be removed by urinary excretion
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www.ohf.org