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**TITLE: Disorders of Copper Metabolism**

**PRESENTER:Rachita Nanda**

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**Slide 1:**

Hello, my name is **Dr.Rachita Nanda**. I am an **Additional Professor, Department of Biochemistry, All India Institute of Medical Sciences Raipur, India**. Welcome to this Pearl of Laboratory Medicine on "**Disorders of Copper Metabolism**."

**Slide 2:**

The outline of this presentation is as follows. I will cover copper metabolism followed by some disorders of copper metabolism which will be discussed under pathogenesis, clinical features and diagnosis and treatment.

**Slide 3:**

Copper is an important trace element of the biological systems with a requirement of 1-2mg/day. Its absorption, distribution and elimination are highly regulated. It has multitude functions that starts with its ability to adopt two different redox states—in the oxidized (Cu2+) and reduced (Cu+) serving as a catalytic cofactor in redox chemistry like the cytochrome C oxidase in electron transport chain to provide energy. Other enzymes like superoxide dismutase, a free radical scavenger, ferroxidase for iron absorption, dopamine beta hydroxylase for catecholamine synthesis, monoamine oxidase for catecholamine metabolism, tyrosinase for pigment formation and lysyl

oxidase for tissue integrity also depend on copper. There are also some copper dependent transcription factors that regulate gene expression.

**Slide 4:**

Absorption of copper takes place primarily in the duodenum, the absorption not being influenced by the amount of copper stored in the body. The copper transporter receptor 1 (CTR1) is responsible for uptake of copper by intestinal epithelial cells. A major portion (25-60%) is transported out by ATP7A (a transmembrane copper-transporting P-type ATPase, mutated in Menke's disease) to the basolateral surface, into the portal circulation and to liver. This protein is expressed in the intestine and other tissues but not the liver. In other tissues, ATP7A supplies copper to the organelles to synthesize certain enzymes like peptidyl-α-monooxygenase, tyrosinase and lysyl oxidase. The rest of the copper gets bound to metallothionein and is lost in feces during shedding of intestinal epithelial cells with a small amount also lost in urine. In the liver, the copper can be stored, or carried, by binding to apoceruloplasmin as ceruloplasmin to sytemic circulation or is excreted into intestinal lumen after biliary excretion.

**Slide 5:**

The copper enters the liver through CTR1 and then gets complexed with metallothionein or glutathione Copper is helped by various chaperone proteins and is carried to specific proteins and organelles for synthesis of certain cellular enzymes. It also gets incorporated to apoceruloplasmin at the trans-Golgi apparatus with the help of ATP7B transmembrane copper-transporting P-type ATPase protein, (a product of Wilson's disease gene) to form ceruloplasmin that enters into systemic circulation. In response to increased hepatocellular copper content, the ATP7B relocates to a vesicular compartment close to the canalicular membrane to cause biliary copper excretion. Whenever, the copper concentration is low, ATP7B incorporates copper into apoceruloplasmin to generate holoceruloplasmin but at high copper concentrations it expedites excretion of copper through the biliary system. Thus ATP7B acts as a sensing and monitoring device for copper within hepatocytes. The copper-glutathione complex

through canalicularmutispecific organ anion transporter, is another minor route of hepatocellular copper excretion.

**Slide 6:**

There are two disorders; inborn errors of copper metabolism which are well characterized but rare in nature. These are: Wilson's disease and Menkes disease. Both these disorders are as a result of mutation in the copper-transporting P-type ATPases; ATP7B and ATP7A, respectively. Further there is a group of disorders that are a combination of environmental and genetic causes. These are the infantile and Idiopathic copper toxicosis and Tyrolean infantile cirrhosis.

**Slide 7:**

Wilson's disease is a monogenic autosomal recessive disorder of copper toxicity. The mutation that can result in structural or functional defects in the protein both of which can cause severe disease. As mentioned earlier, the product of this gene, ATP7B is required for synthesis of enzymatically active ceruloplasmin and biliary copper excretion. Impaired function of this protein results in reduced excretion of copper leading to pathologic accumulation in liver. The excess copper in liver exceeds the storage capacity of metallothionein, so copper gets deposited in lysosomes which through oxidative stress and free radicals mediate the damage of liver mitochondria resulting in hepatocyte inflammation and damage. This spills over to plasma with toxicity of the extrahepatic organs including brain, cornea and erythrocytes.

**Slide 8:**

The major presentation in Wilson's disease involves hepatic, neurological and psychiatric, ophthalmological and renal systems. Hepatic presentation like fatigue, anorexia, abdominal pain, nausea, jaundice (self-limiting), severe coagulopathy and encephalopathy are observed due to hepatic steatosis, inflammation, fibrosis, cirrhosis and liver failure. Coombs negative hemolytic anemia results from direct toxic effects of copper on red cell membranes. Copper gets deposited in brain primarily in the basal

ganglia which results in symptoms of Parkinsonism like dysarthria, clumsiness, tremor, drooling, gait disturbances, mask like facies and deterioration of writing. Sometimes psychiatric symptoms like changes in personality, depression and anxiety can be observed. The ophthalmologic symptoms show the presence of copper and sulfur rich granules in the Descemet membrane of cornea giving a golden brown ring or a greenish discoloration in the limbus, known as the Kayser-Fleischer Ring, under slit lamp.The associated renal symptoms can be microscopic hematuria or Fanconi syndrome.

**Slide 9 and Slide 10:**

The Leipzig criteria published by the European Association for the Study of the Liver and validated in adults and children, should be used for the diagnosis of Wilson's disease, considers both the clinical and laboratory data to establish the diagnosis. The clinical data includes presence of Kayser-Fleischer rings and neurological manifestations. The laboratory testing includes ceruloplasmin, urine copper, hepatic copper, coombs test for hemolytic anemia and molecular testing for ATP7B mutations. When a score of 1-2 is achieved it excludes Wilson's disease while a score of 3 suggests further evaluation. The diagnosis is established after achieving at least 4 points.

**Slide 11:**

There are more than 600 mutations of ATP7B gene. The majority of mutations are missense mutations, with few mutations for specific populations. It is very rare to find large deletions. When mutations at both alleles are identified in the proband, accurate diagnosis of Wilson disease can be made. Direct genetic testing, focusing on sequence analysis of *ATP7B* mutation hotspots are the mode of detection of mutations.

**Slide 12:**

Copper chelators like penicillamine; promote excretion of copper in urine. Inhibits accumulation of copper in hepatocellular lysosomes and solubilizes copper for mobilization from these particles. It also inhibits collagen cross-linking and has some

immunosuppressive properties. Trientine, or triethylenetetraminedihydrochloride, is the usual second-line treatment for patients who are intolerant of d-penicillamine. Trientine chelates copper and increases urinary copper excretion and may interfere with intestinal absorption of copper. Zinc salts increases metallothionein protein synthesis in intestine to trap the copper deposited in the intestinal cells so that it gets excreted in feces. As penicillamine is a pyridoxine antagonist, supplementation with this vitamin is recommended. In extreme cases of liver failure, there may be a need of liver transplantation. However, early detection and with good compliance to treatment, there is excellent prognosis of this disease.

**Slide 13:**

Menkes disease is an X-linked inherited recessive disease caused by mutation of ATP7A gene. This gene is expressed in all tissues except the liver. ATP7A protein along with the ATP7B protein function as sensors of intracellular copper concentration. ATP7A acts as an intracellular pump to transport copper into the trans-Golgi network for incorporation into copper requiring enzymes including dopamine beta–hydroxylase and also mediates copper removal from cells.

**Slide 14:**

Mutations of this gene are associated with loss of functions, with defective copper transport across intestine, brain and placenta. In the intestine there is accumulation of excess copper due to failure of copper efflux, resulting in systemic copper insufficiency and reduced activities of carious copper dependent enzymes. At the blood brain barrier copper efflux is hampered resulting in accumulation of copper in these cells and therefore failure of copper uptake in the brain.

**Slide 15:**

The associated clinical features include hypothermia, neuronal degeneration in cerebral hemispheres, cerebellum and spinocerebellar tracts with associated demyelination, mental retardation, failure to thrive. Cherubic face with abnormalities in hair which is

steely and depigmented (scalp hair called kinky or steel wool). Skeletal changes due to connective tissue defect like flaring and cupping of ribs and lateral and medial spur formation on the proximal or distal femoral and humoralmetaphyses with bone fractures. Low levels are found in, for example, plasma, liver, and brain, while excessive accumulation has been documented in other tissues, such as intestinal mucosa, kidney and placenta.

**Slide 16:**

There is no single sensitive and specific test for diagnosing copper related diseases. The serum copper and ceruloplasmin are decreased which is reliable only beyond 6 weeks of life. Decreased hepatic copper and increased urine copper excretion is observed. Early detection can be facilitated by elevated dihydroxyphenylalanine(DOPA) and diydroxyphenylacetic acid(DOPAC) with reduced neurochemicals like epinephrine and nor-epinephrine and of their metabolites. Defective function of dopamine beta hydroxylase also leads to increased urine ratios of HVA/VMA. There is increased urine excretion of β2- microglobulin.

**Slide 17:**

Diverse mutations are seen in ATP7A in Menkes disease. It is advisable that copper histidinate be administered as early as possible. In absence of early diagnosis and treatment prognosis is bad.

**Slide 18:**

A milder allelic variant of Menkes disease, the occipital horn syndrome, occurs due to a molecular defect of leaky splice junction or hyomorphic missense mutations in ATP7A gene, with 20-30% residual activity. Symptoms observed include dysautonomia related to dopamine-beta-hydroxylase deficiency and connective tissue.

**Slide 19:**

Infantile and childhood copper toxicosis syndrome encompasses copper toxicosis occurring in infants and young children seen in many parts of the world, and is usually associated with high levels of hepatic copper, caused by high concentrations of copper in drinking water or food. Indian Childhood Cirrhosis(ICC), restricted to the Indian Subcontinent occurs in infants the infants who were fed milk stored in brass or copper containers. Characterized by increased hepatic, urinary and serum copper, with rapidly progressive liver cirrhosis. This has become rare due to education of families for not cooking,eating and serving in copper and brass vessels.Idiopathic copper toxicity is similar to ICC and has been named as ICC like disease but caused by high concentrations of copper in drinking water or food like the contaminated spring water in endemic Tyrolean infantile cirrhosis. It is also suspected to be a rare disorder due to unidentified genetic defect.

**Slide 20:**

Promising modes of detection and therapy herald future directions. New born screening program is being developed to enable early detection and hence early treatment of patients for Menkes disease and Occipital horn syndrome. For therapeutic challenge adenovirus mediated gene therapy and liver directed gene therapy for Wilsons disease is being tried to enable a healthy life.

**Slide 21: References**

1. Schilsky ML. Wilson disease and related disorders.Handb Liver Dis. 2018;253–68.
2. Cox DW, Roberts EA. Chapter 76 – Wilson Disease [Internet]. Eleventh E. Sleisenger and Fordtran's Gastrointestinal and Liver Disease.Elsevier Inc.; 2016. 1270-1279.e2 p. Available from: http://www.sciencedirect.com/science/article/pii/B9781455746927000764
3. Mulligan C, Bronstein JM. Wilson Disease: An Overview and Approach to Management. NeurolClin [Internet]. 2020;38(2):417–32. Available from: https://doi.org/10.1016/j.ncl.2020.01.005

4. Bandmann O, Weiss KH, Kaler SG. Wilson's disease and other neurological copper disorders. Lancet Neurol [Internet]. 2015;14(1):103–13. Available from: http://dx.doi.org/10.1016/S1474-4422(14)70190-5
5. Hordyjewska A, Popiołek Ł, Kocot J. The many "faces" of copper in medicine and treatment. BioMetals. 2014;27(4):611–21.
6. Langley A, Dameron CT. Copper and anesthesia: Clinical relevance and management of copper related disorders. Anesthesiol Res Pract. 2013;2013.
7. Kaler SG, Packman S. Inherited Disorders of Human Copper Metabolism [Internet]. Seventh Ed. Reference Module in Biomedical Sciences.Elsevier Inc.; 2014. 413– 443 p. Available from: https://doi.org/10.1016/B978-0-12-812535-9.00011-X

**Slide 22: Disclosures**

**Slide 23: Thank You from www.TraineeCouncil.org**

Thank you for joining me on this Pearl of Laboratory Medicine on "**Disorders of Copper Metabolism**."

## QUESTION BANK TEMPLATE

| Field | Instructions | |
|---|---|---|
| Stem | Write one question<br>*Refer to Guide for Presentersfor guidance (Page 5)* | Which of the following serve as sensor of intracellular copper concentration? |

| | | |
|---|---|---|
| Responses | Provide 5 responses<br>*Refer to Guide for Presentersfor guidance (Page 5)* | a. Metallothionein<br>b. CTR1<br>c. Ceruloplasmin<br>d. ATP7A and ATP7B<br>e. Tyrosinase |
| Answer | Indicate one correct response | d. |
| Discussion | Provide a discussion of the correct response with main points explaining why it is the best choice | ATP7A and ATP7B proteins are key in transporting copper out from intestine and liver. |
| Source(s) | Provide the source(s) of information for further study<br>*Refer to Guide for Presenters for full citation formatting (Page 3)* | 1. Bandmann O, Weiss KH, Kaler S. Wilsons disease and other neurological copper disorders. Lancet Neurol 2015;14:103-13. |
| Difficulty | Select one level of difficulty:<br>*Easy, intermediate, advanced* | Intermediate |
| Category | Select one category (*Refer to list in Guide for Presenters- Page 6)* | Chemistry |
| Sub-category | Select one sub-category (*Refer to list in Guide for Presenters- Page 6)* | General clinical chemistry |
| Keywords | Include at least 1-2 keywords<br>*Keywords should describe a subtopic to the sub-category selected. Examples include, thyroid, electrolytes, diabetes, pregnancy, etc.* | Copper metabolism, trace elements |
| **Field** | **Instructions** | |
| Stem | Write one question<br>*Refer to Guide for Presentersfor guidance (Page 5)* | Supplementation of zinc increases copper excretion through which route? |

| | | |
|---|---|---|
| Responses | Provide 5 responses<br>*Refer to Guide for Presentersfor guidance (Page 5)* | a. Urine<br>b. Bile<br>c. Stool<br>d. Sweat<br>e. Saliva |
| Answer | Indicate one correct response | c. |
| Discussion | Provide a discussion of the correct response with main points explaining why it is the best choice | Zinc increases metallothionein protein synthesis in intestine to trap the copper deposited in the instestinal cells so that it gets excreted in feces. |
| Source(s) | Provide the source(s) of information for further study<br>*Refer to Guide for Presentersfor full citation formatting (Page 3)* | 1. ChenJ, JiangY Shi H, et al. The molecular mechanisms of copper metabolism and its roles in human diseases. Pflugers Arch-Eur J Physio 2020;1415-1429. https://doi.org/10.1007/s00424-020-02412-2 |
| Difficulty | Select one level of difficulty:<br>*Easy, intermediate, advanced* | Intermediate |
| Category | Select one category (*Refer to list in Guide for Presenters- Page 6)* | Clinical Chemistry |
| Sub-category | Select one sub-category (*Refer to list in Guide for Presenters- Page 6)* | General clinical chemistry |
| Keywords | Include at least 1-2 keywords<br>*Keywords should describe a subtopic to the sub-category selected. Examples include, thyroid, electrolytes, diabetes, pregnancy, etc.* | Copper absorption, trace elements |

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Title: Disorders of copper metabolism- **Point by point list of changes**

| **Comments** | **Action taken** |
|---|---|
| **Slides 4&5:** | |
| Text is quite small, can it be enlarged for improved visibility? | Increased font size |
| These two slides include several important notes which are not discussed including ATP7A, Ceruloplasmin synthesis, ATP7B, etc. These are discussed in later slides, if not discussed here it may be best to remove for clarity or briefly mention how they are involved in absorption and excretion. | Mentioned in figure and text |
| Incorporating a copper symbol throughout this figure would be helpful to indicate where/how the copper is moved. | Inserted copper symbol |
| It would also be helpful if arrow size reflected major and minor pathways (small arrows for minor pathways, large for major). | Thicker and thinner arrows placed |

| | |
|---|---|
| I'm unclear from the bile canaliculi why there are 2 exit arrows, I believe this should be just one? | Changed to one |
| It would be helpful to label the polarization of the hepatocyte. | Labeling of polarization done |
| In these two slides I think a line or two more discussion about the function of ATP7A and B would be very helpful in setting up later slides. | Mentioned |
| **Slide 6** | |
| It maybe helpful to define that both Wilson's and Menkes diseases result from mutations in the copper-transporting P-type ATPases, or indicating that both are copper transport disorders. These are both also inborn errors of copper metabolism. The other disorders are reasonably characterized and are thought to be caused a combination of environmental and genetic causes. | Mentioned in text of slide 6 |
| **Slide 7** | |
| Rather than this slide with words it seems that using slide 5 again with these items noted on it could be memorable and informative. (eg – show many copper ions in the liver spilling into the plasma and perhaps add a cornea and brain to the figure with copper ions in them?) | Changes done by using slide 5 again and modifications made in figure as suggested |
| Also a great place to show and talk about the lysosomes addressed in slide 8 | Added in figure and text |
| **Slide 8** | |
| Likely worth mentioning hemolytic anemia as well in this slide since Coombs neg hemolytic anemia is part of the Leipzig criteria. | Both mentioned in slide and text |
| **Slide 9** | |
| Can you list the Leipzig criteria tests here? (Kayser-Fleischer rings, neurologic symptoms, ceruloplasmin, coombs-neg hemolytic anemia, liver copper, urinary copper, mutation analysis). To fit this the title could be shortened to "Biochemical parameters". Please note the spelling of "Wilson's disease" | Leipzig criteria tests listed, title shortened and spelling corrected |
| **Slide 10** | |
| The term "proband" here may be confusing for trainees and can likely be left out. | 'Proband' left out |
| **Slide 13** | |
| As with Wilson's disease, I think this could use a modified version of slide 4. | Slide modified along with text |
| **Slide 14** | |
| It should be somewhere stated that these are the parameters for Menkes disease – in the text and/or slide | Mentioned |
| **Slide 17** | |
| There have been many descriptions of ICC-like disease with all demonstrating hepatotoxic injury following dietary copper in genetically susceptible children, yes ICC itself is, by definition, restricted to India but the same has been described elsewhere and is worth noting as perhaps ICC and ICC-like diseases. | Mentioned |
| **Question Bank** | |

| | |
|---|---|
| Create questions that are directly addressed by the presentation and clearly worded. | Modified the two questions in question bank. |
| | |