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Challenges and Dilemmas in pediatric hepatic Wilson's Disease

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Abstract

Wilson disease is an autosomal recessive disorder affecting the ATP7B gene located on chromosome 13q. This leads to copper deposition in various organs, most importantly in the liver and brain. The genetic mutations are vast, well reported in the West but poorly documented in developing countries. Hence the diagnosis is made with a constellation of clinico-laboratory parameters which have significant overlap with other liver diseases and often pose a significant dilemma for clinicians. Diagnostic scoring systems are not fool-proof. The availability and affordability of chelators in developing countries impact the drug compliance of patients. While D-penicillamine is a potent drug, its side effects lead to drug discontinuation. Trientine is cost-prohibitive in developing countries. There is no single test to assess the adequacy of chelation. Exchangeable urinary copper is an essential upcoming diagnostic and prognostic tool. In the presence of cirrhosis, hypersplenism clouds the assessment of myelosuppression of drugs. Similarly, it may be difficult to distinguish disease tubulopathy from drug-induced glomerulonephritis. Neurological worsening due to chelators may appear similar to disease progression. Presentation as fulminant hepatic failure requires rapid workup. There is a limited window of opportunity to salvage these patients with the help of plasmapheresis and other liver-assisted devices. This review addresses the challenges and clinical dilemmas faced at beside in developing countries.

INTRODUCTION

Introduction: Wilson disease (WD) is a genetic disorder of copper metabolism, with an autosomal recessive mode of inheritance. The estimated prevalence of WD is approximately 30 per million population.[1] Among all causes of hepatobiliary disorders in children, in a 3.5-year study period, in a tertiary care centre in India, WD accounted for 7.6% of total cases.[2] It is important to make an early diagnosis for the prevention of disease progression and the identification of pre-symptomatic cases. This paper addresses the management difficulties in Wilson disease in terms of the identification of varied atypical clinical presentations, diagnostic dilemmas, and pharmacotherapy-related problems.

Pathogenesis: Figure 1 depicts the etiopathogenesis of WD. WD is caused by a mutation in the ATP7B gene, located on chromosome no. 13 Long arm, (13q14.3). This gene codes for an ATPase (a metal-transporting P-type adenosine triphosphatase), which is membrane-bound and mediates trans-Golgi migration of copper within the hepatocyte and its subsequent excretion into the biliary canaliculi.[3] Copper ($Cu^{+)}$ from the diet enters the hepatocyte *via* the copper transported (CTR1) located in the apical membrane. Then it binds to Atox1 (antioxidant protein1) and is then handed over to ATPase (gene product of ATP7B) for its transport into the trans-Golgi network (TGN).[4] Ceruloplasmin has two functions- i. it transports copper to the trans-Golgi network (TGN) where apoceruloplasmin binds to 8 atoms of copper to form holo-ceruloplasmin ii. in copper loaded condition, ATP7B goes to the endocytic vesicles to mediate copper excretion into the bile canaliculi.[5] Thus, in normal conditions, ATP7B is present in the trans-Golgi network and helps in the synthesis of holo-ceruloplasmin. But, in the copper-excess state, the protein moves towards the canalicular membrane to promote copper excretion.[5] Apo-ceruloplasmin is the inactive form of ceruloplasmin and is less stable. In the absence of this transporter, apoceruloplasmin degrades, and non-TGN-transported copper accumulates in the liver and gets bound to metallothioneins (endogenous chelators). When the copper binding capacity of metallothioneins is

overwhelmed, the free copper spills into the lysosomes and causes free radical-mediated cell damage. Ultimately, this free copper comes to the peripheral circulation, where it is weakly bound to albumin and gets deposited in other tissues (brain, kidneys, cornea) (Figure 1). Apoceruloplasmin is a plasma protein and carrier of copper, mainly produced by the liver. In normal physiology, after copper reaches the liver *via* the portal circulation, 20% of copper is re-excreted into the GI tract *via* bile and the remaining 80% is transported to the periphery, bound to ceruloplasmin (Apoceruloplasmin + copper = holo-ceruloplasmin). However, in WD, holoceruloplasmin is reduced, and free copper (non-ceruloplasmin bound copper) is elevated, which leads to high urinary excretion of copper. [6,7] Hepatic involvement is the most common presentation in children with WD, as liver is the major organ responsible for copper homeostasis. Initially, copper binds with metallothionein and accumulates in lysosomes and progressively causes mitochondrial damage leading to hepatic steatosis, fibrosis, and cirrhosis. Subsequently, non-ceruloplasmin-bound excess and toxic copper leaks into the blood and accumulates in the other tissues like brain, leading to neurological manifestations, which is the commonest presentation in older children and adults.

Diagnostic difficulties: Hepatic Wilson disease may present as acute hepatitis, persistent or intermittent elevations of transaminases, organomegaly, fluctuating or worsening jaundice, resembling autoimmune hepatitis, fatty liver, acute liver failure or as decompensated CLD. [6,8,9] Other major presentations of WD include neurological presentation with extrapyramidal symptoms and psychiatric manifestations in the form of tremors, dystonia, dysarthria, gait disturbances, and psychiatric manifestations, like behavioral abnormalities and psychiatric manifestations, like bipolar affective disorder and psychosis with cognitive decline. It is hypothesized that it is because of copper which gets deposited in the basal ganglia by penetrating through the choroid plexus (fenestrated endothelium).[4] Minor presentations include renal tubular acidosis presenting with microscopic hematuria, osseo-muscular involvement in the form of

pathological fractures, arthralgia, proximal muscle weakness.), and importantly haematological presentation in the form of acute fulminant liver failure with Coomb's negative haemolytic anemia.

To make the diagnosis of Wilson disease, we use the presence of a Kayser-Fleischer (K-F) ring on slit lamp exam, 24-hour urine copper of >100 micrograms (mcg) per day, and ceruloplasmin <20 milligrams/decilitre (mg/dL) commonly. These biochemical parameters are not to be interpreted in isolation; it is prudent to remember various factors that affect them. Table 1 summarizes all the diagnostic tests and their interpretation. Table 2 enlists various diagnostic tests used in suspected WD patients and for family screening.

Ceruloplasmin: Apo-ceruloplasmin combines with copper and is secreted from the liver in the form of holo-ceruloplasmin. Serum ceruloplasmin is measured by two methods; either enzymatically by measuring its copper-dependent ferroxidase activity (holo-ceruloplasmin) or by antibody-based tests such as radioimmunoassay, radial immunodiffusion, or nephelometry.[10] The enzymatic method measures holo-ceruloplasmin, whereas immunologic assays measure both apo ceruloplasmin and holo-ceruloplasmin and thus can overestimate ceruloplasmin levels.[10,11] It has been suggested that the enzymatic method is better than the immunologic method to diagnose Wilson's disease. [12] In those patients with impaired synthetic functions due to causes other than WD, the specificity of both these tests dropped, but the enzymatic test had better specificity as compared to immunologic assays (84.5% vs. 68.9%).[12] However, in routine practice, immunological assays are more readily available, rapid and commonly used. The normal value of ceruloplasmin ranges between 20-40 mg/dL. Ceruloplasmin level < 20 mg/dL with the presence of KF ring is considered consistent with WD.[8] Ceruloplasmin< 5 mg/dL is strongly suggestive of WD.[6] Also, ceruloplasmin level <14 mg/dL has a 100% positive predictive value for the diagnosis of WD and a value <5mg/dL is strongly suggestive of WD.[8,13] Serum ceruloplasmin is also an acute phase reactant, and may be falsely elevated in presence of sepsis and it may be falsely low in conditions like fulminant hepatic failure of any etiology, advanced liver disease,

celiac disease and aceruloplasminemia, nephrotic syndrome, and 20% of heterozygotes.[10,11,14]

24-hour urine copper excretion: It is another indirect marker of non-ceruloplasmin-bound copper, which is elevated in WD. 24-hour urine copper excretion of more than 100 mcg per day is diagnostic of Wilson's disease.[15] However, certain other conditions like autoimmune hepatitis, chronic cholestatic liver disease and acute fulminant liver failure of any aetiology may give rise to high 24-hour urine copper excretion.[10] Basal copper excretion may be low in children and asymptomatic siblings, thus a normal value doesn't rule out WD. The test may give rise to false results in case of improper urine collection and copper contamination if kept in certain types of containers. 24-hour urine copper of more than 40 mcg per day may indicate WD in asymptomatic individuals. In addition to making a diagnosis, this is also a valuable test in monitoring the adequacy of chelation, a 24-hour urine copper of 200-500 mcg per day is considered the target.[8]

Liver biopsy and Hepatic copper: On liver biopsy, early changes include steatosis, and fibrosis and late changes include changes of chronic active hepatitis with bridging fibrosis, occasionally resembling autoimmune hepatitis and, Mallory-Denk bodies.[16] Copper staining is demonstrated by orcein, which stains the copper-associated proteins, and rhodamine which stains the elemental copper. Copper-associated proteins may be elevated in various causes of advanced cholestatic liver disease (e.g., Primary biliary cirrhosis or Chronic hepatitis B) other than Wilson's disease, thus histochemical staining for copper is not specific for Wilson's disease.[17] Also, negative histochemical staining for copper does not rule out the possibility of WD.[18] Copper deposition also depends on the stage of disease, early in the disease, copper staining is diffuse in the cytoplasm in the periportal region, and later in the disease, it is mainly seen within the lysosomes of hepatocytes in the periphery of the regenerating nodules. This pattern of distribution suggests that the cytoplasmic distribution leads to hepatocyte damage while copper in the lysosomes is likely to be less toxic. Initial cytoplasmic deposition of copper is due to the high affinity of the sulfhydryl-rich proteins (metallothionines) present in the cytoplasm as compared to lysosomes.[19]Hepatic copper of more than 250 mcg/gram

(gm) dry weight of the liver is strongly suggestive of copper deposition in the liver but it may be also found in any advanced liver disease. Liver copper <50mcg/gm dry weight almost excludes the diagnosis of WD.[8,20] Also, the regional variation due to regenerative nodules and tissue fibrosis is to be kept in mind while interpreting hepatic copper.[10]

Serum copper: It is important to measure the serum copper simultaneously while measuring ceruloplasmin, as it helps in the calculation of the "free copper" also known as "non-ceruloplasmin bound copper (NCC)". Total serum copper comprises of holo-ceruloplasmin (70%), albumin-bound (20%), peptide-bound, and free copper. Holo-ceruloplasmin level is low in WD. So, in most patients of WD, serum copper is low, unless there is a massive release of free copper from the liver due to liver necrosis.[21] This free copper, which is not bound to ceruloplasmin is known as "non-ceruloplasmin bound copper", is elevated in WD and is toxic. Serum copper is estimated by either atomic absorption or emission spectrometry or by inductively coupled plasma mass spectrometry (ICP-MS) methods.[22] Total serum copper (in µg/L)= serum copper (in µmol/L) x 63.5.[10] The normal range of serum copper is 14- 24 µmol/L (90- 150 mg/dL).[11] Holo-ceruloplasmin contains 3.15 µg of copper per mg of ceruloplasmin, hence, ceruloplasmin-bound copper may be calculated as serum ceruloplasmin (mg/dL) x 3.15 (µg copper per mg ceruloplasmin).[11] Free copper or non-ceruloplasmin bound copper (NCC) = [Serum copper-ceruloplasmin bound copper]. Non-ceruloplasmin-bound copper has been proposed as a diagnostic test for WD.[23] In normal individuals, the NCC (free copper) is 10-15 µg/dL.[3] In untreated patients, it is >25µg/dL.[3] Estimation of NCC depends on the methods used to estimate serum ceruloplasmin and copper levels. Falsely high ceruloplasmin calculated by immunological tests may lead to the calculation of a falsely low or negative value of free copper. In normal conditions in WD, serum copper is low. In acute fulminant WD, due to a massive release of copper from the liver, free copper may rise up to ten times and can cause intravascular hemolysis.[23] NCC is not validated for diagnosis of WD but is helpful in monitoring treatment response. With adequate chelation, the NCC decreases

to < 5  . Among patients who are on chelation therapy, the target 24-hour urine copper excretion is 200-500mcg/day. However, patients with poor compliance to chelators can also have low 24-hour urine copper excretion as there is adequate chelator in the circulation to leach out copper. Such patients can be differentiated by estimating the NCC. Those with high NCC >15mg/dL have poor compliance while those with NCC <5 mg/dL are over-chelated.[10]

Exchangeable copper (CuEx) and Relative exchangeable copper (REC): Exchangeable copper is measured by adding EDTA to the serum sample. As EDTA is a copper chelator, it binds with copper, and EDTA-bound copper is measured as exchangeable copper. It measures copper bound to albumin, and other amino acids, which easily gets exchanged with EDTA (copper-chelating agents).[21] Unlike NCC, it doesn't depend on serum ceruloplasmin levels. Normal values for CuEx are between 0.62 and 1.15 µmol/L.[24] Poujois *et al* demonstrated that CuEx is normal to moderately increased in WD, but elevated in those with acute fulminant WD and WD with extrahepatic involvement. In patients with extra-hepatic manifestations, CuEx was the only biological marker to be positively correlated with the neurologic disease burden (assessed by Unified Wilson Disease Rating Score). CuEx determination is, in consequence, useful when diagnosing WD with a value >2.08 µmol/L, and is indicative of the severity of the extra-hepatic involvement. However, CuEx did not indicate the severity of liver damage. CuEx is an interesting experimental biomarker but needs to be interpreted with caution, especially in WD patients with hepatic manifestation.[25] In this study, it was postulated that there may be a threshold concentration of CuEx above which organs like the brain and eyes (and possibly others such as kidneys and heart) may be affected by copper overload.[25] Relative exchangeable copper (REC) is the ratio of exchangeable copper to total serum copper. Balkhi *et al* showed that REC >18.5% can be used to diagnose WD with a sensitivity and specificity of 100%.[26] Trocello *et al* showed that REC can also be used to diagnose WD in asymptomatic siblings, by

taking REC cut-off of 15%, it can distinguish asymptomatic WD from heterozygotes accurately.[27]

Kayser Fleischer (KF) ring: It represents copper deposition of copper in Descemet's membrane of the cornea, visible on slit lamp examination, it is pathognomonic of WD. It is not specific to Wilson's disease and may be found in various cholestatic conditions. It is present in 44-62% of hepatic WD and 95% of neurological WD.[3] KF-like rings have been described in primary biliary cirrhosis, autoimmune hepatitis and severe cholestasis where the total bilirubin is >10g/dL. They are called bilirubin rings and disappear with the resolution of jaundice.[28] It is important to note that the appearance of the KF ring is not related to disease severity and its resolution may take variable time, months to years.[29,30] Anterior segment-optical coherence tomography (AS-OCT) can also be used to diagnose the KF ring, where the KF ring is visualized as an intense reflectivity in Descemet's membrane and it may have better accuracy in diagnosing KF ring as compared to slit-lamp examination.[31] Broniek-Kowalik *et al* showed that AS-OCT detected copper deposition in 15 additional patients of WD, in whom the KF ring was not seen in slit lamp examination.[32]

Coomb's negative hemolytic anemia with a disproportionate elevation of AST as compared to ALT is suggestive of acute fulminant WD. The ratio of AST/ALT >2.2 and ALP/total bilirubin ratio <4, when combined is suggestive of acute fulminant Wilson disease with a sensitivity and specificity of 100%.[33] Alkaline phosphatases are metalloenzymes and zinc is a co-factor. In fulminant WD, during the period of massive release of copper into circulation, copper may compete with zinc for incorporation into alkaline phosphatase apoenzymes. Copper-containing enzyme released into the circulation would have little or no enzymatic activity, and serum alkaline phosphatase values would be low.[34]

Neurological Imaging: The face of the giant panda sign, which is caused by the normal intensity of red nuclei, preservation of signal intensity in the lateral portion of pars reticulata of substantia nigra and hypointensity in the superior colliculus with hyperintense surrounding tegmentum, is considered pathognomonic of neurological

WD.[35] This finding has been shown to reverse with chelation.[36,37] Other findings include lesions in the putamen, globus pallidus, caudate , thalamus, midbrain, pons, and cerebellum as well as cortical atrophy and white matter changes. Usually, the lesions are hyperintense in T2 and hypointense in T1 weighted images. MRI changes correlate with disease severity. Diffuse atrophy of the brain is one of the most common features.[35] MRI findings are universal in symptomatic patients and occasionally reported in pre-symptomatic patients.[38]

Genetic analysis: Lastly, genetic analysis can be done in patients having ambiguity in diagnosis or for sibling screening. More than 600 pathogenic variants have been identified.[39] Most common mutations are single-nucleotide missense and nonsense mutations.[39] There is no genotype-phenotype correlation. Siblings may have varied presentations.[40] On screening the proband, the chances of a sibling being affected is 25% while either parent being affected is 0.5%.[6]

Leipzig score: A diagnostic score named Leipzig score was proposed in 2001, which took into account various clinical, and biochemical, parameters to diagnose WD. A Leipzig score of more than 4 is highly suggestive of WD, while a score of 2-3 would merit further investigations and a score of less than 1 makes the diagnosis of WD unlikely.[41] There are practical difficulties in implementing the Leipzig score in developing countries. Hepatic copper is not universally available and inconsistent in reporting. Genetic testing is cumbersome and region-specific. Most regions do not report the mutations. The d-penicillamine challenge is not reliable. A revised Leipzig score was devised for the Asian setting, which gave importance to family history, greater points for the KF ring and an additional score for a ceruloplasmin level <5mg/dL.[6]

Family Screening: First-degree relatives of newly diagnosed patients should be screened by clinical examination, LFT, slit-lamp examination, serum ceruloplasmin, 24-hour urine copper estimation and genetic testing, especially if the mutation is known in the proband. Screening by laboratory tests is usually deferred till 2 years of age though genetic screening is possible.[6] Preservation of the DNA sample of the proband is essential.

Management difficulties: **Differentiating manifestations of WD from chelation-related complications/Atypical symptoms of WD masquerading as chelation-related complications:**

Kidney-related issues in WD: The renal manifestation of WD can be categorized into 1) renal involvement of underlying disease or 2) treatment-related nephrotoxicity. The differentiating features in pathogenesis, laboratory investigations and management of these two conditions are summarized in table 3.The renal involvement of WD can be in the form of renal calculi, hypercalciuria or tubulopathy. Renal manifestations of WD are due to copper deposition in the renal tubular cells. It has been shown that glomerular and tubular functions improve and normalize after starting D-penicillamine.[42] It is vital to differentiate the renal manifestations from the drug-induced glomerulonephritis as the management differs. Renal involvement in WD was first reported by Litin *et al* in 1959 in the form of hypercalciuria.[43] Acute renal failure, which is the most severe form, can be precipitated by massive intravascular hemolysis seen in WD.[44] Renal involvement can manifest with glomerular or non-glomerular (tubular) injury. Aminoaciduria was first detected in patients with Wilson disease by Uzman and Denny-Brown in 1948.[45] Tubular causes are tubulopathies (proximal or distal renal tubular acidosis [RTA]) presenting as renal rickets, polyuria, polydipsia, or macroscopic hematuria (nephrocalcinosis or renal stone), aminoaciduria, glucosuria, proteinuria, hyperphosphaturia, hypercalcemia and defective urinary acidification.[46] Sozeri *et al* in their study of 10 patients of Wilson's disease, for whom excretion of tubular markers could be repeated between 3 mo to 12 years, N-acetyl-β-d-glucosaminidase (NAG),meaning mention full name , beta 2 microglobulin and low molecular weight proteins were higher in the first year, as compared to the subsequent period. This is in contrast to high molecular weight proteinuria, which increases after 1 year of treatment with D-penicillamine.[42] Renal impairment may be mild to severe. It may present as sub-nephrotic or nephrotic range proteinuria, microscopic hematuria, hypercalciuria, renal stones, renal failure, and lastly D-penicillamine-associated glomerulonephritis.[47]

Tubular damage/ RTA: The disease per se can cause glomerular as well as tubular dysfunction, however, tubulopathy is more common. Excess copper deposits in the epithelium of the proximal and distal convoluted tubules, and the thickening of the basement membrane interfere with reabsorptive function of the renal tubules, thereby leading to RTA.[47] Distal, as well as proximal RTA, has been reported in WD. In a cross-sectional study by Kapoor *et al*, done over 1 year duration, 14 out of 25 patients (56%) of WD, had renal tubular acidosis of which 24% (6/25) had distal RTA, 16% (4/25) had mixed RTA, and another 16% (4/25) had proximal RTA.[48] Wolff *et al* described post-mortem kidney biopsies of 5 patients, wherein, on histopathology, glomeruli were normal in all 5 patients, focal areas of degeneration and necrosis of tubular epithelial cells along with copper staining (rubeanic-acid staining) were seen in all patients.[49] Elsas *et al* showed progressive renal impairment in an adolescent with Wilson's disease for whom D-penicillamine was stopped for almost 18 mo (owing to D-penicillamine induced lupus nephritis), with simultaneous renal biopsy showing an increased number of conspicuous electron-dense bodies in the subapical areas of cytoplasm suggestive of metalloprotein complexes.[50] Azizi *et al* described a 9-year-old boy, who first presented with renal colic due to hypercalciuria, only to be diagnosed as WD 1 year later.[51] There are few case reports of children presenting with renal rickets who were finally diagnosed as WD.[52] In a retrospective study of 85 children with WD by Zhuang *et al*, renal impairment was found in 25 (29.4%) of the treatment-naïve patients. Seven of the twenty-five WD patients (28%) had symptoms of renal impairment (one each had acute nephritis, persistent glomerulonephritis, hemolytic uremic syndrome, and 2 had nephrotic syndrome).[47] Five children had evidence of the glomerular cause of hematuria while the remaining two had non-glomerular hematuria. Twelve of the 25 (48%) had proteinuria, 14/25 (56%) had hematuria, and 5/25 (20%) had both proteinuria and hematuria, 4 /25 (16%) had glucosuria.[47] Thus, by characterizing the type of hematuria (glomerular or non-glomerular) and proteinuria (low molecular weight or high molecular weight), the site of renal involvement can be ascertained. In the treatment -naïve patients, albuminuria with elevated serum creatinine, and low

creatinine clearance suggest glomerular involvement. On the other hand, glycosuria, LMW proteinuria, non-glomerular hematuria, an increase in NAG and beta2 microglobulin, nephrocalcinosis, hypercalciuria, and non-anion gap metabolic acidosis indicate tubular involvement.

Glomerular involvement can be due to i) copper deposition in the mesangium, leading to membranoproliferative glomerulonephritis ii) IgA nephropathy caused by IgA deposits in the glomerulus, due to the loss of scavenging capacity of the liver iii) chelation (D-penicillamine) induced. IgA nephropathy: Gunduz *et al* have reported a 13-year-old boy who presented with nephritic syndrome after 4 mo of diagnosis of Wilson disease, and was diagnosed as membranoproliferative glomerulonephritis on renal biopsy with positive immunofluorescence for IgA, suggestive of IgA nephropathy.[53] Similar case of IgA vasculitis is described by Acharya *et al* in an 11-year-old boy with Wilson disease with F2 fibrosis on liver biopsy (METAVIR staging), who presented with palpable purpura without any arthritis or gastrointestinal involvement.[54] He was diagnosed with IgA nephropathy and mild tubular epithelial degeneration and atrophy on renal biopsy and had complete resolution of the rash and achievement of normal renal function within 3 to 6 mo of chelation. This is similar to primary IgA nephropathy, abnormally glycosylated IgA1 form large soluble IgA1 immune complexes by combining with IgG and IgA, which deposit in the mesangium and lead to mesangial injury. Clinical and histopathological changes may reverse after liver transplantation.

Treatment-related nephrotoxicity: D-penicillamine-induced nephrotic syndrome was first reported by Fellers and Shahidi in 1959.[55] D-penicillamine can cause inhibition of enzymes required for collagen synthesis, thus can damage the glomerular basement membrane and reduce GFR or it can act as a hapten and induce the formation of immune complexes leading to membranous glomerulonephritis.[56] D-penicillamine-induced proteinuria occurs in <10% of patients of Wilson disease and usually begins after 1 year of treatment.[57,58] The spectrum of D-penicillamine-induced nephrotoxicity ranges from membranous glomerulonephritis, tubule-interstitial disease, crescentic glomerulonephritis, Goodpasture's syndrome and renal-limited vasculitis.[59,60]

Proteinuria is the most common manifestation and is the first abnormality to be detected followed by progressive renal disease if D-penicillamine is not stopped.[60] In a case series reported by Sternlieb, all the patients were started on racemic penicillamine, the clinical features resembled nephrotic syndrome, 3 of whom improved following discontinuation of drugs, and another 5 required steroids and there was a recurrence of nephrotic syndrome in all 3 patients in whom penicillamine was re-started while 2 of them didn't have any recurrence after they were started on D isomer of penicillamine.[61] Initially, the racemic mixture of penicillamine was incriminated in the causation of nephrotic syndrome, however, later on, it was shown that even the D-isomer can cause proteinuria. Since 1960, the D-isomer of penicillamine has been available and was approved by the FDA in 1963.[61] Histological examination shows membranous nephropathy (most commonly) minimal change disease or membranoproliferative or rarely crescentic nephropathy.[62,63,64] D-penicillamine causes nephrotoxicity by immunological mechanism, ultrastructural changes include immune-complex deposits, subepithelial deposits and IgG deposits on immunofluorescence.

The incidence of nephrotoxicity due to D-penicillamine was more in conditions unrelated to copper metabolism, *e.g.* rheumatoid arthritis, cystinurias, and scleroderma.[65,66] In a case series of 33 patients, studied by Hall *et al*, who were followed up serially for a mean duration of 74 mo, it was found that the onset of proteinuria peaked after the first 6 mo, with 27/33 developing proteinuria within 1 year of starting D-penicillamine.[59] There are reports to show that the time period from exposure of D-penicillamine to proteinuria may range from a few weeks to years.[60] Siafakas *et al* report a 12-year-old boy who presented with nephrotic syndrome (nephrotic range proteinuria without hematuria, with renal biopsy showing minimal change disease and a negative immunofluorescence study) after 2 wk of D-penicillamine and showed resolution of proteinuria in 3 wk, after being started on steroids.[67] There are studies to show that proteinuria increases even after stoppage of D-penicillamine (within 1-5 mo), and then gradually improves over a few months, which can be explained by Wilson disease-related tubulopathy, as it improves over time with chelation.[42,60,63] In the study by Hall

et al the median first and last creatinine clearance almost remained the same, and none went into renal failure or required corticosteroids. The spontaneous resolution was seen within 2 -32 mo of the stoppage of the drug.[59,63] According to Hall *et al*, among 33 patients of patients on D-penicillamine who had proteinuria, the number of patients in whom proteinuria resolved by 6, 12 and 18 mo were 12 (36%), 21(63%), 29 (88%)respectively.[59] Renal biopsies done in all patients, 29/33 showed ultrastructural changes characteristic of membranous glomerulonephritis with IgG deposits and complement deposits on immunofluorescence, the remaining 4 patients' biopsies showed mesangial proliferation without any deposition of immunoglobulins or complement on immunofluorescence.[59] The severity of proteinuria is not related to the duration or dose of d-penicillamine or HLA typing.[59] In a retrospective study of 63 patients who developed nephrotic syndrome after being started on D-penicillamine, (75% had rheumatoid arthritis,10% had Wilson disease), the mean duration of D-pen exposure to proteinuria was 7.6 (±3.90) months and mean duration of drug exposure until the diagnosis of nephrotic syndrome was 11.9 (± 18.8) months. Fifty-five percent of them had membranous glomerulonephritis and 27% had minimal change disease.[63]

In a study of 8 patients of rheumatoid arthritis, who developed proteinuria while on D-penicillamine of variable duration, ranging from 3 to 48 months and dosage ranging from 250mg/day to 1.2gm per day, 5 patients had scanty subepithelial deposits (spikes), 1 had plenty of sub-epithelial deposits, 2 had associated findings of glomerulosclerosis, interstitial scarring and, on immunofluorescence, 3 showed a positive granular pattern of fluorescence along basement membrane for IgG and C3.[68] After stopping D-penicillamine, proteinuria resolved in 3, progressive proteinuria was seen in one patient, mild proteinuria persisted in 4 others, with the persistence of proteinuria being seen up to 27 mo after stoppage of D-penicillamine.[68] Some studies have been done to see whether D-penicillamine can be resumed after the resolution of proteinuria.[69] In one of the studies in rheumatoid arthritis patients, D-penicillamine was re-introduced after 3 mo of resolution of proteinuria at a low dose of 50mg/day, escalated to a maximum of 250mg/day, showed that none of them had a relapse of proteinuria. However, this low

dose is not enough to treat WD. In another study by Bacon *et al* in 14 patients of rheumatoid arthritis, 3 developed proteinuria, and 11 developed nephrotic syndrome in a mean duration of 7.5 mo of starting D-penicillamine.[64] Renal biopsies showed a picture similar to membranous nephropathy on light microscopy, granular deposits on immunofluorescence (IF), positive for IgG and C3 and sub-epithelial dense deposits on electron microscopy, even after 3- 12 mo of stoppage of treatment. A renal biopsy done soon after stopping D-penicillamine showed effacement of podocytes with discrete electron-dense deposits in the subepithelial region and positive fine granular immunofluorescence with IgG and C3. When the biopsy was repeated after 6 mo of discontinuation, it showed normal foot processes with occasional dense deposits and focal weak immunofluorescence with IgG and C3. Among 3 patients in whom proteinuria persisted at a high level even after stoppage of the drug, biopsy done at 6, 8,12 mo of discontinuing D-penicillamine, electron microscopy still showed discrete but much smaller electron-dense deposits, and brilliant fluorescence with IgG.[64] D-penicillamine-induced glomerulonephritis and Goodpasture syndrome have also been described.[70]

To summarize, diagnosis of D-penicillamine-induced nephropathy can be made with routine urine microscopy, 24-hour urine estimation for proteinuria, and type of proteinuria (tubular or glomerular), and if feasible, histopathological and electron microscopic examination (renal biopsy) especially in the first 18 mo. Membranous nephropathy is the most common nephropathy caused by D-penicillamine.[59,63,68] Serum MPLA2R antibodies (an immunofluorescence test) can be done as a supportive test. These antibodies are prevalent in primary membranous glomerulonephritis in up to 68.5% of cases and their absence on the face of renal biopsy picture of membranous nephropathy suggests the secondary cause of membranous glomerulonephritis.[71] Senthil *et al* reported nephrotic syndrome in a 24-year-old lady, a case WD on D-penicillamine for 18 mo. Renal biopsy showed membranous nephropathy with positive immunofluorescence for IgG and C3, and with negative serum auto-antibodies to M-type phospholipase A2 receptor ($MPLA_2R$, which is usually seen in primary

membranous nephropathy), thus supporting the diagnosis of secondary membranous glomerulonephritis.[72]

Management of D-penicillamine nephrotoxicity includes stoppage of the drug and use of trientine as an alternative. In the study done by Neild *et al*, glucocorticoids had no effect on the natural history of nephropathy.[63] In another case series by De Silva, among 35 patients who developed proteinuria while on D-penicillamine, 60% developed nephrotic syndrome (NS). Among NS patients, D-penicillamine was continued for 62%, stopped at a variable interval, and it was shown that within 4 mo of stopping D-penicillamine, proteinuria decreased to <2g/day and 12 mo after stopping D-penicillamine, proteinuria was 0-0.3g/day.[73] AASLD recommends prompt withdrawal of D-penicillamine immediately.

Haematological manifestations: These include Coomb's negative hemolytic anaemia and acute renal failure, D-penicillamine-induced myelosuppression and lastly hypersplenism (splenomegaly).

Acute fulminant WD: Acute fulminant Wilson disease usually presents with acute intravascular hemolysis, which is due to free copper-mediated damage to the RBC membranes, mortality is 95%.[74] It needs to be tackled with plasmapheresis while awaiting liver transplantation.[75]

Drug-induced cytopenia: The rate of myelotoxicity varies from D-penicillamine 0 to 7% and it is one of the most fatal adverse effects.[76] It has been hypothesized that marrow toxicity could be of two types: the first being an idiosyncratic reaction, leading to cytopenias occurring within the first year of treatment, and the other being a dose-dependent gradual fall.[77] Day *et al* showed that of the 69 patients of rheumatoid arthritis, who were on D-penicillamine for more than 1 year, 15 (21.7%) had developed dose-dependent hematological adverse effects; while those who were on <500 mg per day, had no cytopenias.[78] However in another case series of 10 patients of rheumatic arthritis with D-penicillamine induced myelosuppression, 7 had sudden onset myelosuppression, of these, 6 patients died, and the remaining showed gradual recovery of marrow over 1 year.[79] These studies are to be interpreted keeping in mind 2

points; first, both the studies were in rheumatoid arthritis patients, myelosuppression was documented even with low dose of D-penicillamine (in contrast to WD, where higher dose of D-penicillamine is required), secondly, these patients didn't have concomitant hypersplenism (to confound the picture), thereby, cytopenia could easily be attributed to D-penicillamine, leading to faster decision making in terms of drug-discontinuation. Once myelosuppression is there, D-penicillamine is to be stopped. European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) has suggested monitoring blood counts initially for 1-3 mo and later 3-6 moly.[80] Table 4 enlists the risk factors that may complicate/mimic drug toxicity features.

Neurologic symptoms: Neurological symptoms include dystonia, dysarthria, gait abnormalities, and tremors, with dysarthria being the most common.[8] Other manifestations include abnormal gait, musculoskeletal symptoms, seizures, behavioral problems, drooling of saliva, and chorea.[81] Pediatric patients present with hepatic WD whereas neurological manifestations are more common among adults.[82] Neurological manifestations of WD are due to high concentrations of copper in brain tissue as well in CSF and anti-copper therapy leads to improvement in symptoms.[83] It is essential to characterize the disease extent in order to monitor the evolution of symptoms carefully. It is important to identify the subtle neurologic manifestations at the time of diagnosis as it has a bearing on choosing the medical therapy. Multiple studies have shown that neurological worsening on starting chelation is more commonly seen in those having neurological manifestations, within 1-3 mo of starting therapy. In one of the early studies, Brewer *et al* showed that neurological worsening post-D-penicillamine occurred in 50% of neurologic WD, with more than half of the patients showing deterioration within 4 wk of chelation.[84] Ranjan *et al* performed an MRI brain at the time of neurological worsening after D-penicillamine and showed the appearance of new lesions in MRI in white matter, thalamus, pons, and midbrain which showed diffusion restriction with simultaneous blood investigations revealed increased free serum copper, malondialdehyde and reduced glutathione.[85] Litwin *et al* showed neurological worsening in 11% of neuro-hepatic WD in a mean time of 2.3 ± 1.9 mo from the time of

treatment initiation independent of the type of chelation used.[86] One hypothesis proposed is that there is a sudden release of free copper from the liver, which enters the blood-brain barrier and causes brain damage.[84] Second hypothesis is that the free copper is generated by the chelators within the brain tissue itself. This was substantiated by an increase in free copper concentration in serum and brain tissue, within 3 days of starting on D-penicillamine, measured by ultrafiltration in an animal model of WD. It was noted that at the time of the increase in brain tissue-free copper, there was a fall in protein-bound copper in the brain tissue. A simultaneous increase in immunofluorescent staining of ATP7A (copper transporting protein in neurons) and CTR1 (mediates copper uptake in mammalian tissue) in the cortex and basal ganglia, and not in the blood-brain barrier, suggests that the free copper is generated probably by mobilization of copper within the brain parenchymal cells and not *via* peripherally released free copper entry *via* the blood-brain barrier.[87] The expression of ATP7A present in the subcellular level to mediate biliary excretion of copper (in this animal model) correlated with the free copper in the cortex and basal ganglia. The third hypothesis proposed by Miki *et al* is that the penicillamine-copper complexes that are generated are non-toxic, so, shouldn't cause tissue damage. However, these complexes can catalyze oxidation of the ghost membranes, due to changes in the redox potential of copper, Cu^{2+} to Cu^{1+}), thereby causing neurological symptoms.[88]

Treatment: Once the diagnosis is established, the patient is to be started on lifelong pharmacotherapy, depending on the stage of the disease. WD patients can be broadly classified into three subtypes; symptomatic WD, asymptomatic WD with active disease (on biochemical, histological or imaging findings), and pre-symptomatic WD (mostly those detected on family screening). Those with symptomatic or asymptomatic active disease need to be started on chelation. Patients who are asymptomatic or without active disease can be treated with lower maintenance doses of chelators or zinc alone. It takes around 6-18 mo of consistent chelation therapy to improve the organ function and then patients can be shifted to a lower dose of chelator and zinc.[8]

Table 5 summarizes all the drugs used in the treatment of WD, their mechanism of action, dose, storage and important side effects. There is a fundamental difference in the mechanism of action of chelators and zinc. Chelators decrease the copper load in the body by chelating the copper in the liver, enterocytes and extrahepatic circulation. Whereas zinc induces the synthesis of metallothioneins, which in turn bind to copper and sequester it in the enterocyte (copper is lost as the enterocytes shed off). Thus, the action of zinc is slow. In scenarios, which demand fast and immediate action, for example, advanced liver disease, chelators are a better choice than zinc.

D-penicillamine: D-penicillamine (D-3β,3β-dimethylcysteine; C5H11NO2S) has been the first line of treatment, since its discovery in 1956. It acts by chelating divalent metal ions using its thiol (-SH) group and forming a water-soluble complex which is excreted in urine. It chelates extracellular copper and mediates its excretion in urine. Also, it chelates the intracellular copper from tissue complexes.[81] In WD, the excess free copper in the cytoplasm of hepatocytes, after saturating the metallothioneins, deposits in the lysosomes and causes free radicle-mediated cell damage. D-penicillamine, trientine, as well as zinc, increase the expression of metallothioneins, which bind to copper ions. Metallothionein is a cysteine-rich protein that is an endogenous chelator of copper.[5,81] D-penicillamine also solubilizes the copper deposited in the lysosomes, without much affecting the metallothionein-bound copper and reduces cell damage.[89] Thus, D-penicillamine causes the excretion of copper but can also lead to the sequestration of free intracellular copper with metallothioneins.[5] D-penicillamine also has an anti-inflammatory effect, which is why it is a second-line drug in rheumatic diseases.[90] The standard dose of D-penicillamine is 20mg/kg/day in 2-3 divided doses, it is usually started at a dose of 1000-1500mg/day, and after liver functions improve it can be reduced to a maintenance dose of 10-15mg/kg/day or 750-1000mg/day.[8] Around 40-70% of D-penicillamine is absorbed in the proximal intestine, and peak plasma concentration is reached within 1-3 h. After long-standing treatment, it is eliminated within 4-6 days (slow pool of drug bound reversibly to the tissue). Eighty percent of the drug circulates in bound form with D-penicillamine and the rest in free form. The drug

is eliminated *via* the kidneys.[91] In neurohepatic WD, the dictum is to start at a low dose and increase slowly. Indian National Association for the Study of the Liver (INASL) recommends starting D-penicillamine at 250mg on alternate days and increasing by 250mg every 2-3 wk, until the maximum dose of 1000-1500mg per day, while keeping a watch on neurological symptoms.[6] As D-penicillamine causes depletion of pyridoxine by direct effect, pyridoxine needs to be supplemented at a dose of 25-40mg per day. D-penicillamine should be given either 1 hour before or 2 h after meals, as it may get bound to the copper in the diet before its absorption into the intestine.[92] Antacids and iron significantly decrease its absorption.[8] Brewer *et al* showed that fecal excretion of copper is significantly less with penicillamine and zinc combination than with zinc alone.[93]

Adverse effects of D-penicillamine are acute (early onset) and late-onset (table 5): Acute adverse effects include hypersensitivity reaction, in the form of fever, rash, urticaria, arthralgia, proteinuria and leukopenia, usually within the initial 3 wk of starting D-penicillamine, seen in up to 15% of patients.[6,94] Delayed hypersensitivity include pemphigus, lupus-like syndrome.[6] Other delayed adverse effects include various organ systems, such as hematological (both idiosyncratic and dose-dependent marrow suppression), renal (membranous glomerulonephritis, and neurological (worsening of neurological symptoms). Renal and hematological adverse effects have already been discussed in the article earlier. In case of early hypersensitivity reactions, the drug is to be stopped immediately. In case of isolated dermatological involvement, it may be re-started under steroid cover.[6] Other dermatological adverse effects include dermatopathies due to elastic fibre abnormalities e.g elastosis perforans serpiginosa, and pseudo-pseudoxanthoma elasticum. About 15-30% of patients develop these adverse effects due to high doses and a long duration of drug intake. These skin changes are caused due to inhibition of the aldol crosslinking of tropocollagen and take months to years to manifest (as it takes a long time for new weakened collagen to be synthesized).[95] Penicillamine-induced autoimmune dermatoses include pemphigus, epidermolysis bullosa and lupus-erythematosus-like syndrome. Immune-mediated

toxicities like Goodpasture syndrome, SLE, and nephrotic syndrome warrant immediate drug withdrawal.[8,58]

In some cases, paradoxical neurological worsening is seen after starting d-penicillamine, likely due to peripheral mobilization of copper from the liver to the bloodstream and its subsequent deposition in CNS, seen in 11-50%. [84,86,96,97] Large-scale comparative studies should be done to study the effect of chelators on neurological WD by correlating the dose of chelators with MRI changes and copper transporting molecules by *in vivo* studies. Reversal of neurologic deterioration was seen in only 50% of those affected as D-penicillamine was being continued.[84] In another retrospective study, partial/complete reversal was seen in 53% (8/15) and a further partial response in 13% (2/15) of cases during 9.2 ± 5.2 mo.[86] Thus, the solution to this problem is to start at a low dose and increase it gradually.

Trientine: It is also known as triethylenetetramine (TETA). It is an alternative to D-penicillamine for chelation, particularly for those who do not tolerate D-penicillamine. One molecule of trientine combines with copper in a 1:1 ratio to form a stable complex, which is excreted in the urine. Trientine dihydrochloride is the oral preparation, which requires cold storage at 2^0-8^0 celsius to maintain its stability, failure to maintain cold chain is a common cause of drug discontinuation.[98] Since 2018, trientine is also available as trientine tetrahydrochloride. Woimant *et al* showed that there was no difference in efficacy of the two drugs and in terms of adverse effects, there was a case of recurrence of lupus erythematosus-like syndrome with trientine dihydrochloride.[99] Trientine tetrahydrochloride is stable at room temperature, has slightly more rapid absorption and higher bioavailability and greater systemic exposure. Also, it is a cheaper alternative. It is poorly absorbed with a bioavailability of 8-30% It reaches maximum plasma concentration in 1-3 h.[100,101] Plasma concentration of trientine is significantly reduced when given after food.[95] Trientine has fewer side effects. Pancytopenia is rarely caused. It should not be given with oral iron because trientine-iron complexes are toxic. There are no hypersensitivity reactions reported. Other minor adverse effects include hemorrhagic gastritis, loss of taste and nausea, sideroblastic anemia and allergic

rash.[6,102,103]In a multicenter retrospective study of 77 patients with WD, where patients were treated with trientine for an average duration of 8 years (range: 5 mo to 32.5 years), 49.4% had improved hepatic functions, 10.4% of patients remained unchanged, 5.2% showed worsening, and remaining were asymptomatic to begin with. Twenty-two percent of patients had trientine-associated minor adverse effects, with only one patient requiring treatment discontinuation due to anaemia.[104] Neurologic deterioration is reported with trientine, hence, it should be started at a low dose and increased gradually in patients having neurologic manifestations.[8] In a large retrospective cohort of 471 patients (326 receiving D-penicillamine vs. 141 receiving trientine), it was shown that hepatic and neurologic improvement was comparable in either group. Stable neurologic disease as first-line drug was comparable in either group (27.2% for D-penicillamine vs. 20% for trientine). A higher rate of neurologic worsening was reported with trientine as the first-line agent (20% vs. 5.3%).[105] In another randomized control trial among neurologic WD patients, for comparison between tetrathiomolybdate, 6/23 (26%) in trientine had neurologic worsening as compared to 1/23 in the tetrathiomolybdate group.[106] The dose is 750-1000mg per day or 20mg/kg/day in 3 divided doses. It is to be given 1 hr before or 2 hr after food.[100]

Zinc: It induces synthesis of metallothioneins, and thereby promotes copper-binding to metallothionein in the enterocyte and ultimately hinders its absorption (as copper is lost when the enterocyte is shed). It is a slow de-coppering agent, it decreases the copper absorption but doesn't lead to a sudden massive increase in free copper, hence, it is of choice in neurological WD. However, because of the slow reduction in copper, it is not suitable for treating florid symptoms.[8] Disease may show progression in the initial few months, because of its slow onset of action.[106] In the first pediatric study, in which trientine was used as the initial chelator, it was shown that once adequate chelation was achieved, zinc combination therapy and subsequently zinc monotherapy maintained normal ALT/AST levels.[102] Among those having weight <50kg, it is to be given in a dose of 25mg thrice a day and in those weighing >50kg, 50mg thrice a day.[6] Various zinc preparations are available; zinc acetate, zinc gluconate, and zinc sulfate. There is no

statistically significant difference in various zinc preparations in terms of improvement of liver function.[107] There are few adverse effects, gastric irritation is the most common side effect, in 30-40% of patients. It can also cause an asymptomatic elevation in amylase and lipase.[8] It is recommended to use zinc in pre-symptomatic patients or in the maintenance phase of treatment in symptomatic patients.[8] In a systematic review and meta-analysis, zinc showed better improvement as compared to D-penicillamine in neurologic WD, however, there was no difference in hepatic WD. Also, the incidence of adverse effects and neurologic deterioration was higher with D-penicillamine as compared to zinc (RR:2.42, 95%CI: 1.20%–4.88%; *P* = 0.014) and RR: 1.96, 95%CI: 1.31%–2.93%; *P* = 0.001, respectively).[108]

Ammonium Tetra-thiomolybdate: It acts by forming a tripartite complex with copper and protein, which is stable. It binds the copper present in food and prevents its absorption. When given without food, it is absorbed into the blood and forms complexes with copper bound to albumin, thus preventing its deposition in various organs.[109] Also, it enters the blood-brain barrier and enters neuronal cells.[110] In an open-label study, 55 neurologic WD were treated with tetrathiomolybdate (120-410mg for 8 wk), followed by zinc maintenance therapy. Only 3.6% (2/55) showed neurologic deterioration. Among the other adverse effects, 5/22 (23%) of treatment-naïve patients had bone marrow suppression and 3/22(14%) had an elevation in liver enzymes and both these adverse effects quickly responded to drug dose reduction.[111] Bone marrow suppression is caused by the depletion of copper and is reversed by decreasing the dose of tetrathiomolybdate. The rise in the liver enzymes could be due to the mobilization of hepatic copper (from hepatic pools including metallothioneins) in a heavily copper-loaded liver, which is reversible on dose reduction.[111] In a subsequent double-blind RCT between tetrathiomolybdate (120 mg per day) and trientine (1000 mg/day) among 48 patients of neurologic WD, as mentioned above, tetrathiomolybdate was better than trientine, only 4% worsened neurologically *vs* 26% in trientine arm.[106] Ammonium tetrathiomolybdate is unstable for routine use, bis-choline tetrathiomolybdate is a more stable complex and has a better availability. Recently, bis-choline tetrathiomolybdate

underwent a phase II trial among 28 neurologic WD, where it was given for a span of 24 wk, with the target to achieve a primary end-point of normal value of non-ceruloplasmin bound copper $_{corrected}$ (NCC $_{corrected}$ stands for NCC corrected for copper contained in tetrathiomolybdate-copper-albumin complexes) or achievement of 25% reduction from baseline NCC $_{corrected}$. Twenty-two patients completed the study up to 24 wk and by week 24, 20 (71%) had achieved the primary end-point (treatment success) accompanied by improvement in neurologic status (without any paradoxical neurological worsening, being reported). There were 11 (25%) serious adverse effects which included 6 events of psychiatric disorders in 4 patients, gait disturbance in one patient and two events of raised aminotransferases, one agranulocytosis and a decline in neurologic functioning.[112] Also, Brewer *et al* analysed free copper levels in patients treated with tetrathiomolybdate *vs* trientine and found that the mean free copper was significantly less in the tetrathiomolybdate group at week 4 and week 8 of treatment.[113] Thus, tetrathiomolybdate is a fast copper-lowering agent with minimal adverse effects. The dose recommended is initially 120 mg per day for the first 2 wk, as, 20mg thrice a day with meals and 20mg daily between meals followed by 60mg daily as 10mg thrice a day with meals and 10mg thrice daily between meals.[113] Currently, a phase 3 trial is going on and results are keenly awaited.(NCT03403205).

Comparison between various chelators:

D-penicillamine vs. trientine: In a large retrospective cohort of median follow-up of 13.3 years, both D-penicillamine and trientine were comparable in terms of improvement in hepatic WD (>90% cases) as well as in neurologic WD (>55% cases) while neurologic worsening was more common in trientine group as compared to D-penicillamine group (20% vs. 5.3%, p=.042). Treatment discontinuation due to adverse effects was more common in the D-penicillamine group as compared to trientine (28.8% vs. 7.1%, P = 0.039).[105] In the most recent randomised open-label non-inferiority trial of D-penicillamine *vs* trientine, for maintenance therapy after 1 year of chelation with D-penicillamine, trientine was found non-inferior to Dpenicillamine.[114]

D-penicillamine vs. zinc: There are studies to show that zinc was found to be more effective in ameliorating neurological symptoms in 90% of patients in the zinc arm as compared to 25% alone in the D-penicillamine arm. In a head-to-head comparison between D-penicillamine and zinc, among 67 new patients, of whom the majority were asymptomatic or had neurological disease, 44% discontinued D-penicillamine owing to its adverse effects as compared to 12% in the zinc arm.[116] In Another retrospective study of 288 patients for a period of 17.1 years by Weiss *et al* showed that zinc monotherapy led to 15.9% (14/88) hepatic treatment failure as compared to 1.2% (4/313) in the D-penicillamine group without any statistically significant difference in the adverse effects in either group.[117] Further, these zinc non-responders responded to chelators (either D-penicillamine or trientine). This supports the use of chelators in asymptomatic patients with active disease.

Dhawan et al., Askari et al. and Santos Silva *et al* have shown favourable outcomes with combination therapy but earlier studies by Brewer et al. show no added advantage.[93,118,119,120] A systematic review of 17 articles involving 1056 on combination therapy showed that combination therapies are significantly less effective than individual therapies (47.1 vs.78.6%).[121]

Adequacy of treatment: Treatment targets on chelators: Asymptomatic patients should remain asymptomatic and symptomatic patients should show improvement in liver functions in the initial 6-18 mo.[8] Patients with decompensation might take longer to improve. Patients are to be monitored clinically for symptom improvement/ new symptom onset. Initially, LFTs are to be done, every 3 mo, and thereafter 3-6 mo depending on the disease severity.[8] Adequacy is monitored by monitoring liver function tests and by quantifying 24-hour urine copper excretion or free copper estimation. Free copper estimation, is not a full-proof test if the ceruloplasmin is calculated by the immunologic method as it calculates both apoceruloplasmin and holoceruloplsmin.[6] Also, urinary copper excretion is to be interpreted carefully after

taking proper treatment history. Urinary copper excretion may be high if chelation has been re-started after a period of non-adherence or it can be falsely low, in case of poor drug absorption or inadequate dosing itself. Another way to monitor adequacy is to measure REC.

For de-coppering agents, free copper is a marker of the adequacy of chelation, while for zinc, urine copper levels below a certain cut-off is recommended for good copper control (Table 1).

When to decrease chelation? Transition to maintenance dose or zinc can be used once liver function improves. AASLD recommends a transition to maintenance after clinical and biochemical parameters improve (usually seen after 1 year of therapy). The drug of choice drug could be low-dose chelating agents or full-dose zinc. In a retrospective study of 31 symptomatic hepatic WD who were transitioned from D-penicillamine to zinc (28 due to financial constraints and 3 due to adverse effects), wherein the majority of patients belonged to Child's class C (54%) patients, the average duration of zinc therapy on follow-up was 363 (35–728) weeks. In the Child C cirrhosis group at presentation, who received D-penicillamine for 111(2-230) weeks followed by zinc for 344 (41–652) weeks, 15 had significant improvement in liver function and disease severity scores.[122] In a prospective study of 44 hepatic WD patients, who received D-penicillamine plus zinc combination therapy for more than 2 years, and were in biochemical remission (defined by AST & ALT >1.5 times upper limit of normal (ULN), serum albumin > 3.5 gm/dL & INR < 500 mcg/day & non-ceruloplasmin bound copper (NCC) < 15 mcg/dL), were shifted to zinc monotherapy and biochemical parameters were assessed on follow-up. They showed that 9/44 (20.4%) relapsed till the last follow-up.[123] More prospective studies are needed to establish the correct time of transition and establish guidelines for the same.

CONCLUSION

Conclusion: Wilson's disease is a treatable metabolic liver disease. Early diagnosis is imperative. Wilson's disease has varied manifestations. Certain disease manifestations

need to be differentiated from drug-toxicities, *e.g.*, tubulopathies due to WD vs. D-penicillamine-induced nephrotoxicity and hypersplenism vs. D-penicillamine-induced myelosuppression. Though there are difficulties in making the correct diagnosis, with the help of non-ceruloplasmin bound copper, relative exchangeable copper and newer methods to detect early Kayser-Feisher ring (AS-OCT), diagnosis can be made in resource-limited conditions, where mutational analysis is cost-forbidding. Chelation remains the mainstay of treatment and is to be preferred in active disease whether symptomatic or asymptomatic. Regarding the transition to maintenance therapy, the exact timeline is not yet defined, but depends on the liver function and is to be decided on a case-to-case basis with close follow-up of the copper load in the body. Trientine has been shown to have a good clinical response in a recent clinical trial and its availability as tetrahydrochloride, which is cheaper and doesn't require cold storage gives hope in resource-limited conditions. Bis-choline tetrathiomolybdate is the new addition in the armamentarium, which rapidly decreases the free copper load and is undergoing phase III trials. In fulminant hepatic failure, plasmapheresis has shown (while awaiting liver transplantation) some hope.

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