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# Accepted Manuscript

Wilson's disease: Clinical practice guidelines of the Indian National Association for the study of Liver (INASL), The Indian Society of Pediatric Gastroenterology, Hepatology and Nutrition (ISPGHAN) and the Movement Disorders Society of India (MDSI)

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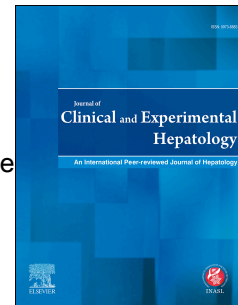
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**Wilson's disease:** Clinical practice guidelines of the Indian National Association for the study of Liver (INASL), The Indian Society of Pediatric Gastroenterology, Hepatology and Nutrition (ISPGHAN) and the Movement Disorders Society of India (MDSI)

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**Abbreviations:** WD: Wilson's disease, Cu:copper, DP: d-penicillamine, TTM: tetrathiomolybdate, AST: aspartate transaminase, ALT: alanine transaminase, ACLF Acute on chronic liver failure, hepatocellular carcinoma, KF: Kayser-Fleischer, MARS: Molecular Absorption Recirculating System, TPE: Total Plasma Exchange, INR:International Normalised ratio, NWI: New Wilson's Index, PELD:Pediatric end stage liver disease, MELD: Model for end stage liver disease, LT:liver transplantation, GAS for Wilson disease: Global Assessment Scale, for Wilson disease

**Key words:** Wilson disease, Wilson's disease scoring, modified Leipzig scoring, rare disease, genetic disorder, review, position paper

ACCEPTED MANUSCRIPT

## **Introduction**

This document is the outcome of a consensus meeting in March 2017 of experts from the Indian National Association for the study of Liver (INASL), The Indian Society of Pediatric Gastroenterology, Hepatology and Nutrition (ISPGHAN) and the Movement Disorders Society of India (MDSI), which represent the major sub specialties involved in the care of Wilson's disease. The need for this was felt as most previous guidelines have either focused on the hepatic aspects (AASLD: American Association for the Study of Liver Diseases, EASL: European Association for the Study of the Liver)<sup>1,2</sup> or pediatric aspects (ESPGHAN: European society of Pediatric Gastroenterology Hepatology and Nutrition)<sup>3</sup> of the disease. Participants at the meeting presented recommendations on specific areas of the disease and then all members voted on them using the nominal voting technique as per standard guidelines.<sup>4</sup> The quality of evidence was adapted from the GRADE (Grades of Recommendation, Assessment, Development and Evaluation) system.<sup>5</sup> The final recommendations were then circulated to all the core group members and updated based on systematic literature search. These guidelines are thus comprehensive and cover all aspects of Wilson's disease (WD). They also reflect the challenges faced by clinicians in resource-limited settings.

## **Nominal group technique of voting**

Hepatologists and neurologists manage WD patients, often with overlapping disease but no joint consensus exists till date. We felt the need for practice guidelines with a holistic management. The process was primarily initiated by the first author (AN). Expert Hepatologists (pediatric and adults) and neurologists were consulted to identify the gaps in literature and difficulties in management practices. Specific questions and areas of interest were identified and distributed to the core members. A two-day deliberation on WD was held on March 2017 in Mumbai. On the first day, various aspects of pathogenesis, management and recent literature review were presented and debated. On the second day, the core members met for formulating the guidelines. AN and RKD moderated the session. Presentations on specifically allotted topics were projected on the power-point by each core member. At the end of each presentation, different viewpoints were debated and finally voted. The statements were recorded. Each presenter was asked to submit the review of literature of their individual assignment by a specific deadline. The information was pooled. The draft was revised several times by AN, MSS and JM with addition and deletion of certain areas. PLK, HD and SS contributed in specific areas including the modified scoring system. The final manuscript was drafted by AN.

1

## **Historical perspective of Wilson's disease**

3 The first possible case of Wilson's disease was described by Frerichs (1861) in a 9-year-old boy with  
 4 movement and speech abnormalities, whose autopsy showed liver cirrhosis. Kayser (1902) followed by  
 5 Fleischer (1903) reported a greenish-brown ring around cornea in patients of suspected multiple  
 6 sclerosis. Wilson (1911), described the disease as familial "progressive lenticular degeneration" in  
 7 association with cirrhosis of the liver and Hall (1921) coined the term "hepatolenticular degeneration,  
 8 while Umpel (1913) demonstrated increased copper (Cu) in liver and basal ganglia in patients with WD.  
 9 Subsequently, Mandelbrot (1948), Scheinberg-Gitlin (1952), and Cartwright (1954) reported increased  
 10 urinary copper, decreased level of ceruloplasmin and increased serum free copper respectively in WD.  
 11 Bearn (1957) first postulated that it was a genetically determined metabolic disease, and Walshe  
 12 (1956)<sup>6</sup> demonstrated treatment success with d-penicillamine (DP) (1956) and trientine (1969)<sup>7</sup>.  
 13 Brewer first documented use of Zinc and tetrathiomolybdate (TTM) in Neuro WD.<sup>8,9</sup>

14

## **Epidemiology of Wilson's disease**

16 WHO estimates that the global prevalence of WD is 1/10,000 to 1/30,000.<sup>10</sup> Clinical prevalence in West  
 17 was estimated 5 decades ago as 5 per million.<sup>11</sup> Since then, it has steadily increased to approximately  
 18 142 per million by modern genetic testing. Some parts of Europe such as Romania and Sardinia report  
 19 highest prevalence (370-885 per million) with 6 mutations accounting for 85% of their cohort.<sup>12</sup>  
 20 Sequencing of *ATP7B* in 1,000 control participants in the UK allowed the frequency of an individual  
 21 carrying two mutant *ATP7B* alleles to be estimated at 1/7,026. In China, the prevalence is estimated as  
 22 5.87 per 100,000. Newborn screening programs show disappointing results. Screening by serum  
 23 ceruloplasmin in children aged 6 months to 9 years yield prevalence of 124 per million in Japan.  
 24 Screening programs recommend 3 years and above as opportune time to detect the disease.<sup>13</sup> In India,  
 25 there are no community-based incidence and prevalence studies of WD. Among pediatric liver  
 26 diseases, WD accounts for 7.6-19.7% in tertiary hepato-biliary centers. Fifteen to twenty new cases of  
 27 WD are registered annually in referral neurology centers.<sup>14</sup>

28

## **Normal copper fluxes and pools**<sup>15,16</sup>

30 The body contains 110 mg of Cu, predominantly in the muscles (28 mg), bones (46 mg) and connective  
 31 tissue. The Cu pool in the musculoskeletal system is in constant exchange with plasma. Plasma contains

approximately 1 mcg/mL, of which 60-95% bound to ceruloplasmin. Ceruloplasmin is a source of Cu for peripheral organs where Cu is an essential cofactor for many enzymes.

The copper circulation is explained in Figure 1. Normal dietary Cu intake is 1.5-5 mg in 24 hours, 50-60% of which is unabsorbed and excreted in faeces. 25-40% is absorbed from duodenum, stored by enterocytes and bound to metallothioneins in a non-toxic form. From this intestinal pool, 75% flows through the portal system with albumin or transcuprein and is taken up by the liver. The remaining 25% is bound to albumin in the circulation. In the liver, 20% of Cu is re-excreted back into the gastrointestinal tract through bile and 80% is transported to the periphery, bound to ceruloplasmin. The biliary excretion is approximately 2.5 mg/d. Near-similar amounts are excreted from other secretions (saliva, gastric, pancreatic and intestinal fluid). These are the endogenous Cu excretions, a large proportion (approximately 80%) is again reabsorbed by the intestinal mucosa. When copper is deficient in the diet, there is enhanced affinity of metallothioneins in enterocytes for copper, thus increasing its absorption and vice versa. Thus, the daily fecal losses are a combination of unabsorbed dietary Cu and small proportion of excreted endogenous Cu amounting to approximately 1.5-4 mg/d. Compared to fecal excretion, urinary Cu excretion is low (10–100 µg/d). The effect of dietary Cu level on urinary Cu excretion is inconsistent, with some studies reporting a small but significant positive relationship while others show no effect.

### **Pathogenesis of Wilson's disease**<sup>17-19,</sup>

WD is an autosomal recessive disorder that affects the ATP7B gene (chromosome 13q) expressed in hepatocytes, kidneys and placenta which encodes for the copper transporting P-type ATPase or copper translocase ATP7B. ATP7B helps in transport of copper to the trans-Golgi network (TGN) and the biliary excretion of copper. Antioxidant protein 1, a copper chaperone helps deliver copper to the six copper binding domains in ATP7B. Binding of copper to ATP7B causes ATP hydrolysis, which supplies energy for transport of copper to the lysosome. Here copper is incorporated into ceruloplasmin, which is then released in the circulation. Levels of copper in the hepatocytes regulate the intracellular distribution and function of ATP7B. With normal copper levels, ATP7B helps in synthesis of cuproproteins like ceruloplasmin. Apo-ceruloplasmin (copper free ceruloplasmin) is less stable in circulation than holo-ceruloplasmin (copper bound ceruloplasmin). When intracellular copper is in excess, ATP7B facilitates its excretion into bile by exocytosis. Mutations in ATP7B result in decreased synthesis of copper bound ceruloplasmin, impaired excretion of copper and increased cytosolic, mitochondrial and nuclear levels of copper.

### ***Copper induced liver injury*<sup>18,19</sup>**

Although not yet fully understood, a few possible mechanisms have been postulated. Increased intracellular copper induces oxidative stress leading to the production of hydroxyl radicals and reduced superoxide dismutase and glutathione which leads to damage of cellular lipids, proteins and nucleic acids. Cardiolipin, on the mitochondrial membrane has been shown to be fragmented by copper induced oxidative stress. Reversible mitochondrial recovery by chelation therapy suggests that the initial damage was possibly due to multivalent interactions/multiprotein cross linking in intermembrane space and condensations within mitochondria and various other organelles. The molecular reasons for variability of clinical symptoms and lack of genotype-phenotype relationship in WD is not yet fully elucidated. Genotype variations, gene-environment interactions and altered activity of other modifier genes have been implicated.

### **Clinical features of Wilson's disease**

Clinical features depend on the predominant organ involved (mainly liver and brain) and the disease has been reported from 3 to 85 years of age. Though copper starts accumulating soon after birth, the disease takes at least 3 years to manifest. Symptoms depend upon the site of deposition of copper in the body. Walshe et al demonstrated an age-phenotypic presentation. Hepatic presentation was seen in younger age groups (<10y: 83%; 10-18y: 52%; >18y: 24%) while neuropsychiatric presentation increased as age advanced (<10y: 17%; 10-18y: 48%; >18y: 74%). Symptom onset to diagnosis was shorter in hepatic (6 months) than neuropsychiatric presentation (18 months).<sup>20</sup> Studies in children from India and Egypt have shown isolated hepatic involvement (20-54%), isolated neurological (8 - 22%), neurohepatic (11 - 36%) , asymptomatic (15 - 35%), and other manifestations (0 - 22%).<sup>21-23</sup> The clinical manifestations are summarized in table 1, while the prevalence from different centres is shown in table 2

### ***Hepatic manifestations***

Liver is the first organ to be involved due to accumulation of copper in WD predominantly manifesting in childhood and is virtually involved in all cases of Wilson's disease.<sup>24</sup> The symptoms related to liver involvement are quite varied and include

1 *Asymptomatic WD*: Has been documented in 3 - 40 % patients in various studies<sup>25,26</sup> These patients  
 2 have incidentally detected hepatomegaly, raised transaminases or are siblings of the index WD patients  
 3 detected on screening. Majority of these cases are identified in first decade of life or in adolescence.

4 *Acute hepatitis*: (10-25 %)<sup>22,27</sup> This mimics acute viral hepatitis, autoimmune hepatitis and drug  
 5 induced liver injury. Jaundice, anorexia, nausea, malaise, fever, pale stools and pain in abdomen are  
 6 often the predominant symptoms. The biochemical investigations show conjugated hyperbilirubinemia,  
 7 raised transaminases and normal or marginally low synthetic functions. It is important to consider WD  
 8 in older children and adolescents with sero-negative acute hepatitis.

9  
 10 *Acute liver failure* (8-20%)<sup>22,28</sup> Predominantly seen in childhood and adolescents. It is usually associated  
 11 with Coomb's negative non immune intravascular hemolysis<sup>29</sup> The presentation mimics acute hepatitis  
 12 but the condition deteriorates rapidly over days to weeks and is often fatal. It results in deep jaundice,  
 13 hemolysis, coagulopathy, ascites, encephalopathy and renal failure. The investigations show very high  
 14 serum bilirubin, mild to moderate rise of liver enzymes, low serum alkaline phosphatase, low serum  
 15 uric acid and defective synthetic functions. This phenomenon has also been reported in patients who  
 16 have stopped the chelation therapy abruptly. Serum alkaline phosphatase /total bilirubin ratio  
 17 <4 and AST:ALT (aspartate ratio >2.2 combined has been described to have a diagnostic sensitivity and  
 18 specificity of 100 %.<sup>30</sup> However, this has not been validated in subsequent studies<sup>31</sup>

19  
 20 *Acute on chronic liver failure (ACLF)* (11-55%)<sup>25,32</sup> In two studies on ACLF from India, WD was the  
 21 underlying chronic liver disease in 42 - 43 % patients with a superadded acute viral hepatitis.<sup>33,34</sup> Viral  
 22 hepatitis is often the acute event that deteriorates underlying hepatic WD and is the first manifestation  
 23 of the disease. There is a prodrome followed by deep jaundice, early onset ascites, encephalopathy and  
 24 coagulopathy rapidly progressing to a multiorgan dysfunction.

25  
 26 *Chronic hepatitis* (10-30%)<sup>35</sup> Seen especially in adolescents and young adults. Nonspecific and  
 27 constitutional symptoms such as fatigue, anorexia, nausea, malaise, may present before onset of  
 28 jaundice and hepatic dysfunction. Associated delayed puberty, amenorrhea, polyarthralgia, may be  
 29 present.

30

**Cirrhosis:** (35%–60%) Patients may present with complications of cirrhosis<sup>22, 25,28</sup> including ascites (spontaneous bacterial peritonitis), encephalopathy, and renal failure (including hepatorenal syndrome) or portal hypertension (variceal bleeding).<sup>36</sup> In adults, although neurological manifestations may dominate the underlying liver is usually cirrhotic. Any young patient more than 3 years of age presenting with cirrhosis should be evaluated for WD as an underlying cause.

**Fatty liver:** Wilson disease should be thought of as differential diagnosis in a child less than 10 years presenting with a fatty liver where Non Alcoholic Fatty Liver Disease is less likely. Various histological and histological series report incidence from 28% - 35.7%.<sup>37,39</sup>

**Cholelithiasis:** Chronic hemolysis leads to mixed gall stones which are cholesterol predominant but also pigmented and patients may present with symptoms/complications of gall stones<sup>35</sup> Copper content of the gall stone is low as these patients have defective excretion of copper into the bile.

**Malignancies:** The development of hepatocellular carcinoma (HCC) in patients with WD is rare compared to other causes of cirrhosis. Retrospective analysis of 363 patients of WD diagnosed in UK and Sweden has shown that 4.2 – 5.3 % develop HCC or cholangiocarcinoma over a 10 – 29 years and 15 % over a 39 year follow up.<sup>39</sup>

### **Neurological manifestations**

Most patients who present with CNS manifestations have liver disease at the time of presentation, though not symptomatic. Contrary to the initial belief that hepatic form is the predominant presentation of WD, over the years published literature has noted that neurological manifestations tend to be more common at presentation, accounting to as high as 60%.<sup>40-42</sup> However, there could be a centre oriented bias in reporting depending on the speciality a patient has been referred to. Patients with neurological presentations tend to be older (second/third decade) and usually have a Kayser-Fleischer (KF) ring (Figure 2). Early or subtle neurological manifestations in young children include deterioration of handwriting and school performance, dysarthria and drooling of saliva. The classical dystonia involving the facial and mandible muscles produce a characteristic “Wilson’s facies”. Wilson’s facies is scored on presence of vacuous smile, open mouth, hypersalivation and dull look, a unique feature which gives an estimate of severity<sup>43</sup> (Figure 3). The presenting neurological symptoms tend to be wide and variable. In a series of 307 patients from India,<sup>44</sup> the common presenting symptoms were

: tremors (31.6%), dysarthria (15.6%), jaundice (12.4%), abnormal gait (8.8%), abdominal distention (7.8%), musculoskeletal symptoms (5.2%), seizures (4.9%), behavioural problems (4.6%), dystonia (3.6%), clumsiness (2.6%), drooling of saliva (2.6%), generalized weakness (2.3%), decreased scholastic performance (1.9%), changed sensorium (1.3%), bleeding symptoms (1.3%), dysphagia (0.9%), chorea (0.3%), and poor vision (0.3%). Over the course of disease, patients tend to develop varied combination of these presenting features.

Primarily the neurological form consists of extrapyramidal manifestations, but not exclusively limited to it.<sup>42</sup> Broadly, the extrapyramidal neurological abnormalities can be classified as: (a) an akinetic- rigid syndrome similar to Parkinson's disease, (b) pseudosclerosis dominated by tremor, (c) ataxia, and (d) a dystonic syndrome. Other major neurological presentations include seizures, and cognitive changes. Seizures are not uncommon and could occur at any stage of the disease with published frequency between 4.2% to 7.5%<sup>42</sup>

#### ***Psycho-behavioural manifestations***

Psycho-behavioural issues are very common in WD, and constitute almost for 1/3rd of presenting symptoms. Almost all the patients will have some form of psychiatric symptoms during the course of their disease.<sup>45</sup> Amongst the psychobehavioral symptomatology, the following remain the core issues: organic dementia, psychosis, psychoneurosis and behavioral disturbances characterized by impulsivity occasionally extending to unlawful behavior.<sup>46</sup> Psychotic symptoms of WD are often undiagnosed and factors like lack of awareness, failure to recognize co-existing neurological features or misinterpretation of such symptoms as side effects of neuroleptic treatment are some of the possible cited reasons. The delay in diagnosis ranges from 1 to 5 years.<sup>47,48</sup> The reported incidence of psychiatric symptoms as the presenting manifestation varies from 2.4% - 20%, and can be categorized into the following groups<sup>49</sup>

***Personality Changes:*** Personality changes include bizarre behaviour, impulsivity occasionally extending to criminal behaviour, disinhibition, irritability, emotionality, decreased threshold for anger and aggression. Bizarre behaviour and irritability are more often associated with bulbar and dystonic features rather than tremors.<sup>42,49</sup>

***Affective Disorders:*** Depression, is the most common psychiatric manifestation and often co-occurs with neurological symptoms, but not with hepatic disease. In the series by Shanmugiah A et al,<sup>50</sup> 18% had bipolar affective disorder, 4% had major depression and 2% had dysthymia. Other affective disorders such as hypomania and mania though rare, have also been reported.

1 *Psychosis*: "Schizophrenia-like" and other forms of psychosis can rarely be the initial  
 2 manifestation, \* but more commonly accompany other neurological features (16-51% )<sup>42, 45,50</sup>  
 3 *Cognitive Impairment*: Mental sub-normality though not described as a feature of WD was found  
 4 in 23% of patients with WD on neuropsychological and intellectual assessment.<sup>52</sup>  
 5 *Others*: Other psychiatric manifestations including substance abuse, catatonia , sexual preoccupation  
 6 and anxiety disorders have also been reported.<sup>53</sup>  
 7 Behavioural symptoms do respond to de-coppering therapy and few may require long term  
 8 symptomatic behavioural pharmacotherapies.

9

### 10 ***Ocular manifestations***

11 Copper accumulates in the Descemet's membrane of the cornea forming the Kayser-Fleischer (KF) ring,  
 12 which is greenish brown in colour (Figure 2). They are always bilateral and are seen in 50-60% of  
 13 hepatic and 95-100% cases of neurological WD.<sup>54</sup> Although sometimes visible to the naked eye, a slit  
 14 lamp examination is necessary for confirmation. They appear sequentially at the  
 15 (upper>lower>medial>lateral) segment of limbus and on chelation therapy disappear in reverse  
 16 direction. KF rings may mimic bile pigment rings seen in the stromal layer of the cornea in advanced  
 17 cholestasis and hence need to be confirmed by an experienced ophthalmologist especially if jaundice is  
 18 present.<sup>55</sup> In a study by Fenu et al, partial or total KF ring resolution was observed in 28%, deterioration  
 19 in 6% and static in the rest of the cohort over 1-3 years of therapy.<sup>56</sup> After liver transplantation, a  
 20 partial decrease in or complete disappearance of the K-F ring has been documented.<sup>57</sup>  
 21 Sunflower-cataract is uncommon (2-17%) and is due to copper deposition in the anterior capsule of the  
 22 lens.<sup>58</sup> These are always associated with KF rings, do not disturb vision and disappear with chelation  
 23 therapy.<sup>54</sup> Abnormal oculo-motor functions are frequently seen on electro-oculography in patients  
 24 with WD but has doubtful clinical significance.

25

### 26 ***Renal manifestations***

27 Tubular injury (copper deposition in epithelium of proximal and distal convoluted tubules) occurs from  
 28 WD (8%) but glomerular injury (10%) is usually a complication of chelation therapy.<sup>54</sup> Tubular injury  
 29 manifests as nephrocalcinosis (microscopic hematuria) and nephrolithiasis (renal colic). Though  
 30 glomerular injury may be due to mesangial deposition of copper,<sup>59</sup> it is more commonly related to the  
 31 d-penicillamine treatment. Hence, at presentation, all cases of WD should be screened for renal  
 32 tubular dysfunction with a urine routine and microscopy, while on follow-up with chelators, proteinuria

should be assessed to detect glomerular injury. In a study of 41 patients with WD (6-37 years of age) who were on treatment d-penicillamine for 0-15 (mean 4.5) years, Sozeri et al have shown that 39% had significant proteinuria.<sup>60</sup> Low molecular weight proteinuria was observed in the first 2 years of treatment, indicating early tubular damage, while high molecular proteinuria suggesting glomerular injury, persisted over longer periods. Children with tubular dysfunction need treatment with bicarbonate in addition to d-penicillamine.<sup>61</sup> D-penicillamine needs to be discontinued (permanently) in those who develop glomerular injury especially if the proteinuria is in the nephrotic range.

### ***Hematological manifestations***

These include Coomb's negative hemolytic anemia and thrombocytopenia with or without hemolysis.<sup>47,54</sup> The sudden release of excess free copper from liver due to hepatocyte necrosis produce oxidative stress on RBCs resulting in hemolysis. The hemolytic anemia may be mild with asymptomatic liver disease or an acute severe form heralding the development of acute liver failure. Patients with advanced liver disease, have abnormal coagulation profile and platelet dysfunction, while splenomegaly from portal hypertension can result in hypersplenism, but none of these are specific for WD.

### ***Osseomuscular manifestations***

Osseomuscular symptoms may rarely be the presenting feature (2%)<sup>40, 42,</sup> or occur during the course of the disease in adults. Osteoporosis (24-88%) osteomalacia (14-35%), spontaneous fractures (9-35%), rickets, osteochondritis dessicans, chondromalacia patellae, premature osteopenia and degenerative arthritis of knees and wrists have been reported in the second and third decade of life.<sup>62,63</sup> Though osteoporosis has been documented on densitometry in 43-67% of patients with WD,<sup>64</sup> most patients are asymptomatic with only radiological abnormalities in the large joints. Except chelation, there is no other specific treatment available for osseo-muscular disorders in WD. Paradoxically, D-penicillamine itself can induce rheumatological disorders like systemic lupus erythematosus, Good-pasture syndrome, myasthenia, dermatomyositis etc.<sup>64</sup> The cause for osseomuscular symptoms is unclear, though symptoms improve with chelation and in vitro studies have documented abnormal copper deposition in cartilage and bones. Complete reversal of joint manifestations following liver transplantation has been documented in a case report from India.<sup>65</sup>

### ***Other manifestations***

Asymptomatic cardiac arrhythmias are quite common in WD. Cardiomyopathy, autonomic dysfunction and cardiac deaths due to copper accumulation in cardiac tissue have occasionally been reported in WD. Kuan et al<sup>66</sup> reported ECG abnormalities in 34%, which include left ventricular hypertrophy, ST wave depression and T wave inversion. Asymptomatic orthostatic hypotension was reported in 19%, abnormal response to Valsalva in 33% and cardiac deaths in two cases (ventricular fibrillation and cardiomyopathy in one each).

Extensor hyperpigmentation due to increased melanin has been reported in 11% of WD patients.<sup>67</sup> Blue lunulae of nails (azure lunulae) and acanthosis nigricans have occasionally been reported in WD.<sup>54</sup> Endocrine abnormalities reported include amenorrhea, gynecomastia and testicular atrophy. Additional features include pancreatic insufficiency, diabetes mellitus, gigantism and hypoparathyroidism<sup>68</sup>

## **CONSENSUS STATEMENTS ON CLINICAL FEATURES**

### **Hepatic manifestations**

1. ***Isolated hepatic involvement is more common in childhood and adolescence than adulthood demonstrating an age-phenotypic nature of the disease. (Strength 1, Level of evidence A).***
2. ***Cirrhosis and portal hypertension is a common presentation in hepatic WD (Strength 1, Level of evidence B).***
3. ***Acute liver failure, acute on chronic liver failure, acute hepatitis, asymptomatic hypertransaminasemia, fatty liver, cholelithiasis and rarely hepatobiliary malignancies are the other manifestations of hepatic WD (Strength 1, Level of evidence B).***
4. ***Concomitant hemolysis is invariably seen in patients of WD presenting with acute liver failure (Strength 1, Level of evidence C).***

### **Neuropsychiatric manifestations**

1. ***Neurological / Neuropsychiatric symptoms can be the sole presenting clinical symptom of Wilson's disease. (Strength 1; Level of Evidence – A)***
2. ***Neuropsychiatric form of Wilson's Disease usually tends to present later than the hepatic form (Strength 1; Level of evidence – B)***

3. *The clinical symptomatology varies significantly affecting various neurological domains, including from mild tremors, dystonia, seizures, Parkinsonism, ataxia, cognitive changes and frank behavioural issues. (Strength 1; Level of evidence -A)*
4. *Any Child / Young adult presenting with neuropsychiatric features, screening for Wilson's disease should be considered. (Strength -1, Level of evidence -A)*

#### Ocular manifestations

1. *KF rings are usually bilateral and present in almost all cases of neurological and more than half of hepatic WD. Slit lamp examination is mandatory for diagnosis (Strength – 1 Level of evidence – B)*
2. *Sunflower cataract is uncommon, even in neurological WD. (Strength – 1 Level of evidence – B)*
3. *Disappearance of KF rings often correlates with adequate chelation, but may take years (Strength -1, Level of evidence –B)*

#### Renal manifestations

1. *Renal tubular dysfunction with nephrocalcinosis (manifesting as microscopic hematuria) is not uncommon in Wilson disease. (Strength-1, Level of evidence –B)*
2. *Children on d-penicillamine should be periodically evaluated for proteinuria to detect drug induced glomerular injury. (Strength-1, Level of evidence -B)*

#### Hematological manifestations

1. *Mild Coombs negative hemolytic anemia can occur in asymptomatic WD. (Strength-2, Level of evidence -C)*
2. *Acute severe hemolysis can be an initial manifestation of acute liver failure related to WD. (Strength-2, Level of evidence -C)*
3. *WD should be suspected in adolescents and young adults with Coombs negative hemolytic anemia. (Strength-2, Level of evidence C)*

#### Other system manifestations

1. *Joint manifestations may be the presenting symptoms of Wilson's disease. Precocious onset degenerative joint disease (osteoarthritis, chondrocalcinosis) in 2<sup>nd</sup> and 3<sup>rd</sup> decade should raise a suspicion of WD. (Strength-2, Level of evidence -B)*
2. *Asymptomatic arrhythmias are common and so a cardiac evaluation may be routinely done in all adult patients. (Strength-1, Level of evidence -B)*

1

## 2 **Diagnosis of Wilson's disease**

3 A combination of serum ceruloplasmin, KF rings and 24-hour urine copper is most commonly used to  
4 diagnose WD. Liver biopsy and estimation of dry copper has been traditionally thought to be of value in  
5 doubtful situations. Extrapyrasidal symptoms associated with KF ring makes the diagnosis of WD  
6 certain. Genetic mutation studies are now available.

7

## 8 ***Serum ceruloplasmin***

9 Ceruloplasmin is a carrier protein produced mainly by the liver for the transport of copper in blood. A  
10 low level in a person with suspected disease may be suspicious of WD. The youngest age for testing  
11 serum ceruloplasmin to diagnose WD is 1 year.<sup>3</sup> Enzymatic assay which measure copper dependent  
12 oxidase activity and antibody dependent immunologic assays such as radioimmunoassay, radial  
13 immunodiffusion, or nephelometry have been used to measure ceruloplasmin levels. Although the  
14 enzymatic method is superior and the preferred method for estimation, the immunological -  
15 nephelometry method is the more commonly available method of measurement.<sup>71,72,70</sup> Normal values  
16 range from 20 to 40 mg/dl. While values <10 mg/dl strongly favour the diagnosis of WD, those between  
17 10-20 mg/dl may be seen in both patients and about 20% of heterozygotes.<sup>73</sup> However, normal values  
18 may be seen in up to a third of patients with WD and may be falsely normal in acute inflammation  
19 (ceruloplasmin is an acute phase reactant). Serum ceruloplasmin is typically lower in neuro-psychiatric  
20 disease compared to liver disease.<sup>74</sup> Ceruloplasmin levels may be decreased in patients with other  
21 causes of cirrhosis, malabsorption and renal disease. Low serum ceruloplasmin levels alone cannot be  
22 relied upon to make a diagnosis of WD, although very low levels below 5 mg/dl are highly suggestive of  
23 WD

## 24 ***24- hour Urinary copper assay***

25 This is a sensitive test that indirectly reflects the serum free copper level. Urine sample must be  
26 collected in a copper free container and the test should be done before chelators are started. It is an  
27 excellent test in symptomatic patients, but may be false negative in those who are asymptomatic.<sup>75, 76</sup>  
28 A level >100 mcg/dl is considered virtually diagnostic, but recent studies have shown that lowering the  
29 cut off levels to 40 mcg/dl for asymptomatic patients increases the sensitivity.<sup>76</sup> The assay is difficult to  
30 perform and should be done only in reliable laboratories

31

### 1 ***D-penicillamine challenge test (PCT)***

2 After a base line 24-hour collection for copper assay, a second 24 hour sample of urine is collected  
3 while the patient is given two 500 mg doses of -D-penicillamine 12 hours apart. Earlier considered to be  
4 a useful test in diagnosis, it is not recommended now in view of the high false positive results.<sup>77</sup>  
5 Lowering the threshold for basal urine copper excretion together with measurement of serum  
6 ceruloplasmin and KF ring may be more useful rather than PCT.<sup>78</sup>

### 8 ***Kayser-Fleischer Rings***

9 Kayser Fleischer (KF) ring should be sought in all patients suspected to have WD.<sup>76</sup> This test may be  
10 negative in asymptomatic siblings or children less than 10 years of age. These are best seen on  
11 conventional slit lamp examination. A hand held slit lamp apparatus may be used in patients who are  
12 not ambulatory.

### 14 ***Serum copper***

15 Measures the total serum copper (ceruloplasmin bound copper + non-ceruloplasmin copper or “free  
16 copper”) Ninety percent of the serum copper is bound to ceruloplasmin.  
17 Total serum copper does not reflect tissue levels and therefore unreliable in diagnosis. Serum free  
18 copper may have a better correlation, especially in patients with acute liver failure. However, the value  
19 of free copper is limited as it relies on the accuracy of tests measuring serum ceruloplasmin and serum  
20 copper.<sup>24</sup>

### 21 ***Serum exchangeable copper***

22 This test corresponds to the copper which is bound to albumin and other peptides. A cut off value of  
23 15% of relative exchangeable copper (namely exchangeable copper to serum copper ratio) has been  
24 reported to have 100% sensitivity and 100% specificity for the diagnosis of WD in adults.<sup>79</sup>

### 25 ***Coomb's negative hemolytic anemia***

26 The presence of significant hemolysis on peripheral smear and a negative Coomb's test in a patient  
27 with acute liver failure makes the diagnosis of Wilson's disease highly likely.<sup>24</sup> Hemolysis contributes to  
28 the elevated serum bilirubin levels and a decrease in hemolysis reflects in a commensurate drop in  
29 serum bilirubin.<sup>2</sup>

1

## 2 ***Liver biopsy and liver copper estimation***

3 Histopathological changes are not specific for WD and range from steatohepatitis, interface hepatitis,  
4 chronic hepatitis with Mallory's hyaline, bridging fibrosis and cirrhosis, most of which are seen with  
5 other liver diseases as well.<sup>80</sup>

6 Although orcein, rhodamine and Timm's stain have been used to identify liver copper, the distribution  
7 is patchy and interpretation is difficult. Positive staining may be seen in diseases associated with  
8 impaired bile secretion.<sup>81</sup> These can be supportive evidence rather than of primary diagnostic  
9 importance.

## 10 ***Liver copper estimation***

11 Often described as gold standard test for WD, it is not easily available and may be fraught with logistic  
12 and quality issues. Biopsy specimens for estimation of copper should be sent in a dry condition in a  
13 copper free container for atomic absorption analysis,<sup>81</sup> although paraffin embedded specimens can  
14 also be analysed for copper. The normal copper content in liver tissue is <50 mcg/g of dry weight while  
15 in WD a level > 250 mcg/g is commonly encountered even in asymptomatic individuals. The copper  
16 level tends to be higher in those with liver dysfunction compared to those with neurological or  
17 asymptomatic disease. Liver copper content is physiologically increased in infancy<sup>82</sup> Uneven distribution  
18 of copper in the liver and low levels of copper in regenerating nodules limits its usefulness in those  
19 with cirrhosis and late stages of WD. In addition, patients with cholestatic liver disorders can have a  
20 high liver copper.<sup>83,84</sup>

21

## 22 ***MRI brain***

23 The presence of neurocognitive features in conjunction with KF ring is often sufficient to make the  
24 diagnosis of WD. Accumulating experience has demonstrated MRI abnormalities to be almost universal  
25 in those with neurological disease. In one study, the MRI features with WD included "Face of giant  
26 panda" (14.3%), tectal plate hyperintensity (75%), central pontine myelinolysis-like abnormalities  
27 (62.5%), and concurrent signal changes in basal ganglia, thalamus, and brainstem (55.3%).<sup>85</sup> These  
28 features when present are virtually pathognomonic of WD.<sup>86</sup> (Figure4)

## 29 ***Family history***

A positive family history of WD (including deaths from compatible liver or neurologic disease) is useful especially when parental consanguinity exists. A recent study from Vellore, India identified consanguinity in 44% of patients with WD.<sup>87</sup>

### ***Genetic studies***

WD is an autosomal recessive disease and in 1993, two separate teams reported mutations in ATP7B gene on chromosome 13. Over 600 mutations have since been described and genetic studies have gradually become part of routine tests in diagnosis. The international consensus diagnostic score (Leipzig score 1993) gave maximum weightage to genetic testing.<sup>88</sup> Within families, the risk of Wilson's disease among siblings of an affected individual ("the proband" or index patient) is 25%, while among the progeny the risk is 0.5%.<sup>89</sup> If ATP7B mutations have been identified in a patient with Wilson's disease, genetic testing to look for these mutations in his / her pre-symptomatic siblings is valuable to differentiate heterozygote carriers from homozygotes or compound heterozygotes for Wilson's disease. Other copper related liver disorders (Indian childhood cirrhosis, atypical copper cirrhosis)<sup>90</sup> also can be differentiated with mutation studies. H1069Q mutation is the commonest mutation seen in over 50% of patients in Western countries is almost non-existent in other countries like India.<sup>91,92</sup> Despite the high rate of consanguinity noted in Indian families that are affected, there is a wide spectrum of mutations observed. This phenomenon can be considered as an 'Indian paradox'.<sup>93</sup> Various studies suggest that p.C271X may be the commonest mutation in patients from Western part of India patients<sup>94</sup> and p.G1101R<sup>95</sup> maybe the commonest mutation in patients from Eastern parts of India with Wilson's disease. C813A was identified in 19% of patients from East India and 12% of patients from South India.<sup>96</sup> Studies have not been able to establish significant correlation between the ATP7B mutations and Wilson's disease phenotype. The large number of ATP7B mutations adds to the complexity of this correlation. It is also recognized that affected siblings within a family (who share the same Wilson's disease genotype) may have different phenotypes.<sup>93</sup> It is still unclear what determines whether a patient with Wilson's disease will develop hepatic or neurological disease or both. Looking for mutations in all 21 exons of ATP7B gene is an expensive and daunting challenge with conventional genetic tests. In addition, many patients with Wilson's disease are compound heterozygotes. Earlier, mutation screening was performed to identify exons likely to harbor mutations as a preliminary step (using single strand conformational polymorphism<sup>97</sup> or conformation sensitive gel electrophoresis\*) and then sequencing only the selected exons as a second step. Technological advances now enable

1 simultaneous analysis for mutations in all 21 exons of ATP7B gene using microarray based tests<sup>98</sup> or  
2 next generation sequencing.<sup>96</sup>

3 Regional centres need to be established where these tests can be done at an affordable cost with a  
4 quick turn - around time. There is also a need for a nationwide network in various countries to  
5 determine the common mutations in each country.

6 Prenatal testing for WD is technically feasible.<sup>97</sup> However, since it is an easily treatable disease, routine  
7 prenatal screening for Wilson's disease does not appear justified.

8

### 9 **Rationale for a new scoring system for diagnosis of WD (modified Leipzig score)**

10 The Leipzig scoring system for diagnosis of WD<sup>88</sup> was modified by the consensus group members by  
11 adding points for a family history or sibling deaths with phenotypes consistent with WD. It was  
12 subsequently validated in 70 patients of proven Wilson's disease. This modified Leipzig score (Table 3)  
13 lays more importance to simple variables that are easily available. As alluded to earlier, liver copper  
14 estimation is not easily available and may have methodical flaws. It was therefore excluded from the  
15 scoring system. Mutational assay may be performed for individuals in whom diagnosis is difficult to  
16 establish by clinical and biochemical testing. Further, unlike in the West there are no common  
17 mutations in India. There are more than 600 that have been identified in other parts of the world  
18 including India. Additional weightage was given to a ceruloplasmin value of <5 mg/dL for higher points  
19 as compared to ceruloplasmin >5 mg/dL. Penicillamine challenge test also has been omitted from the  
20 modified scoring system due to poor yield and inadequate validation.

21

### 22 **Family screening**

23 Family screening of first degree relatives of WD has multiple advantages: 1) it allows early detection of  
24 disease in presymptomatic phase before a devastating course, 2) makes the family wiser and the  
25 physician more prepared and 3) identifies a healthy family member or a heterozygote carrier who can  
26 be a potential donor for living related LT should a diseased member require LT in future. Ideally family  
27 screening should include siblings, parents of affected children and offspring of affected parents. There  
28 is 25% chance of siblings carrying the homozygous disease gene. Screening is deferred till 2 years of age  
29 when presymptomatic patients can be started on chelation therapy. Screening can be initiated earlier if  
30 any stigmata of liver disease or occult warning signs are detected (eg: hepatomegaly or fatty or nodular  
31 liver sonologically in the absence of deranged liver function tests). Histological liver fibrosis as young as

4 month old has been documented.<sup>99</sup> Assessment should include good history, physical examination including slit lamp examination of KF ring, serum ceruloplasmin, liver function tests, 24 hr urinary copper. The role of testing for ATP 7B mutation or haplotype studies assumes importance if the affected individual is alive or sample is stored before death where the specific mutation seen in the index case can be looked for in the family member being screened. Genetic testing is the only reliable method to separate heterozygote from homozygote siblings. There is however difficulty in diagnosing heterozygote carriers with certainty. Though next-generation sequencing (NGS) is promising in detecting both mutant alleles in 95%, the limitations include missing molecular defects outside the coding regions and detecting Variants of Unknown Significance (VUS) which may not be of clinical significance.<sup>3</sup> More recently relative exchangeable copper (REC) appears to be a promising tool for family screening in WD, especially for heterozygous ATP7B carriers who could present with slight biological abnormalities. However further and larger studies are required to validate the same.<sup>100</sup>

#### **CONSENSUS STATEMENTS ON DIAGNOSIS OF WILSON'S DISEASE**

##### **Serum ceruloplasmin**

- 1. Ceruloplasmin value of < 10 mg/dl strongly favors the diagnosis of WD (Strength 1, Level of evidence B)**
- 2. Borderline or normal levels do not exclude the diagnosis and further tests are needed for confirmation WD. (Strength 1, Level of evidence A)**
- 3. Values above normal are unlikely in WD (Strength 2, Level of evidence A)**

##### **24 hour urine copper**

- 1. A basal 24-hour level >100 mcg is a very useful diagnostic test in symptomatic patients (Strength 1, Level of evidence A)**
- 2. Lower levels (>40 mcg) have been recommended especially for asymptomatic siblings but is less specific (Strength 2, Level of evidence A)**
- 3. D-penicillamine challenge test is not standardized, gives high false positive results and should not be used in diagnosis. (Strength 2, Level of evidence A)**

### **KF rings**

- 1. KF ring is highly specific for WD, but its absence does not rule out the diagnosis (Strength: 1, Level of evidence A)**
- 2. While it is almost always present in patients with neuropsychiatric disease only 50-60% of those with liver disease have a KF ring (Strength 1, Level of evidence A)**
- 3. A slit lamp examination by an experienced ophthalmologist is necessary for confirming or ruling out a KF ring (Strength 1, Level of evidence A)**

### **Serum copper**

**Serum total copper has no role in diagnosis of WD. (Strength 1, Level of evidence A)**

### **Hemolytic anemia**

- 1. Any degree of hemolysis in the setting of liver disease that is Coombs negative heightens the suspicion of WD (Strength 2 Level of evidence A)**
- 2. The presence of significant hemolysis in an individual with acute liver failure is almost always due to WD (Strength 2, Level of evidence A)**

### **Liver biopsy and liver copper**

- 1. There are no histopathological features pathognomonic for WD (Strength 2, Level of evidence A)**
- 2. Liver copper estimation has limited usefulness due to nonavailability of the test as well as logistic and quality issues. (Strength 2, Level of evidence A)**
- 3. Histochemical stains for copper may only provide supportive evidence for diagnosis of WD (Strength 2, Level of evidence B)**

### **MRI brain**

**MRI of brain with certain features such as tectal plate hyperintensity are pathognomonic of WD (Strength 2, Level of evidence B)**

### **Family history**

- 1 **1. Positive Family history is an indirect evidence of WD (Strength 2, Level of evidence C)**
- 2 **2. A history of sibling death in patient with features suggestive of WD features makes WD**
- 3 **diagnosis likely although the presence of other associated features strengthens the diagnosis.**
- 4 **(Strength 2, Level of evidence C)**

#### 6 **Genetic studies**

- 7 **1. ATP7B mutation analysis is recommended as a clinical diagnostic test to support the diagnosis**
- 8 **of Wilson's disease in a patient suspected to have Wilson's disease. (Strength - 1, Level of**
- 9 **evidence - B)**
- 10 **2. Genetic testing for Wilson's disease is recommended as a clinical diagnostic test in siblings of**
- 11 **a proband with Wilson's disease, especially if ATP7B mutation is identified in the proband.**
- 12 **(Strength - 1, Level of evidence - B)**
- 13 **3. Routine prenatal diagnosis of Wilson's disease is not recommended. (Strength - 1, Level of**
- 14 **evidence - C)**

#### 15 **Family screening**

16 **Family screening of patients diagnosed with Wilson Disease is recommended: (Strength - 1, Level of**

17 **evidence - A)**

#### 19 **Drug Therapy**

##### 20 ***D-penicillamine (DP)***

21 DP is the preferred standard therapy for WD. It is rapidly absorbed from the intestine, bound to

22 plasma proteins and more than 80% is excreted in the urine.<sup>101</sup> DP binds to copper through

23 disulfide bonds and every gram promotes urinary excretion of 200 mg of copper. It also induces

24 hepatic metallothionein, a cytosolic metal-binding protein that sequesters copper, and renders

25 it non-toxic. Clinical and biochemical improvement usually occurs within a year of treatment,

26 but hepatic synthetic functions may take up to 10 years to normalise.<sup>102</sup> Hepatic Cu levels fall

27 during the first year, but remain above normal for several years. DP chelates several heavy

28 metals, not just copper and has many adverse effects necessitating discontinuation in up to 30%

1 of WD patients (Table 3). Despite the serious adverse effects, DP is still the primary drug for  
 2 management of hepatic WD in view of its time-tested efficacy, easy availability and reasonable  
 3 cost.

4 In neurological WD, a paradoxical worsening of symptoms is occasionally observed after starting DP- .  
 5 While most centres report an incidence of 10%, one study by Brewer et al reported it in 50% of  
 6 patients.<sup>103</sup> DP is therefore started at the lowest possible dose in neurological WD . It may be initiated  
 7 as 250 mg on alternate days. There is no definitive protocol on the rate of dose escalation. While some  
 8 centres increase by 250 mg every two to three weeks, others do so every month, until the maximum of  
 9 1000 to 1500 mg /day in 2 or 3 divided doses is reached. The gradual increase in dose should be done  
 10 under clinical and biochemical monitoring. Similarly rapid re-administration of the drug in patients who  
 11 have stopped therapy for a long time may lead to irreversible neurological changes.<sup>104,105</sup> The  
 12 paradoxical worsening with treatment has been attributed to sudden mobilization of free copper from  
 13 neurological tissues. Neurological improvement is much slower and usually reaches a plateau after  
 14 around 2-3 years of treatment. Lack of improvement in neurological status (e.g. dystonia) may be due  
 15 to permanent neurological damage (putaminal necrosis).

16 The dose of DP is 20mg/kg/day in children, while adults receive 750-1500mg per day in 2-3 divided  
 17 doses on an empty stomach. Food reduces its absorption by 50% and so food should not be given 1  
 18 hour prior to and 2 hours after the drug. Antacids and iron also significantly reduces absorption.  
 19 Pyridoxine deficiency can occur with therapy due to inhibition of pyridoxine kinase enzyme. Hence  
 20 vitamin B6 should be supplemented at a dose of 20 -40 mg in children, pregnant women and patients  
 21 with malnutrition and intercurrent illness.<sup>106</sup> Treatment with DP results in massive and rapid  
 22 cupriuresis (> 1000ug/day) in the initial months of therapy, falling to 200–500ug/day during the  
 23 maintenance period.

24 Significant adverse effects are reported in 10 to 30 % of patients on DP therapy (Table 4).  
 25 Children seem to tolerate the drug better than adults<sup>26,107</sup> In a series of 74 children from UK, the  
 26 reported incidence of side effects was 38 % and in 16 % the drug had to be switched over to trientine.<sup>78</sup>  
 27 Manolaki et al in their series of 54 children have reported a similar incidence of side effects at 16 %  
 28 where the initial treatment with DP had to be discontinued because of the adverse effects.<sup>108</sup> If early  
 29 hypersensitivity reactions occur, the drug should be stopped immediately. If the manifestations are  
 30 only dermatological, DP can be introduced under cover of steroids. Once the skin lesions resolve,  
 31 prednisolone is given initially at a dose of 0.5 mg /kg /day for 2-3 days. DP is then introduced at a low

dose of 5 mg /kg /day and increased gradually while prednisolone is tapered and stopped. However, if the manifestations involve the bone marrow (agranulocytosis, aplastic anemia) or other organ systems, then DP should be stopped. Development of significant proteinuria or glomerulonephritis warrants stoppage of the drug. Direct dose dependent adverse effects of DP is due to the interference with copper –dependent enzyme lysyl oxidase which mediates collagen cross linkage and elastin formation leading to progeria like skin lesions, cutis laxa and elastosis perforans serpiginosa. (EPS)<sup>109</sup> (Figure 5) These usually occur when prolonged treatment is given in large doses.

### ***Trientine***

Trientine (Triethylene-tetramine-2-hydrochloride), is a chelator with a mechanism of action similar to DP, with fewer adverse reactions. The dosage is 750-1500mg/day in 3 divided doses on empty stomach in adults (20mg/kg/day in children). Although trientine has traditionally been used for patients intolerant to DP,<sup>110</sup> recent studies suggest that it can be used as a first line drug.<sup>111,112</sup> There are no clinical trials comparing the relative superiority of one over the other. In most developing countries including India, it is only imported for select patients since it is prohibitively expensive. It is heat sensitive and has to be stored in tightly closed containers between 2-8 degree Celsius. It chelates iron and other heavy metals as well, hence treated subjects should be monitored for iron deficiency. Trientine can also cause paradoxical worsening in neurological WD, Hence, it should be started in low doses and increased slowly similar to DP.

### ***Zinc***

Zn acts by inducing metallothionein in enterocytes which preferentially binds absorbed Cu, sequesters it in the enterocytes and prevents its entry into the portal circulation.<sup>113-115</sup> As the enterocytes are naturally sloughed into the lumen, copper is excreted in the faeces. Zinc also induces metallothionein in hepatocytes and protects against Cu toxicity. Unlike DP and trientine, Zn acts by increasing the faecal excretion of Cu. However, Zn has a slow action and takes much longer to achieve a negative Cu balance as compared to chelation therapy, and hence may be less effective as first line therapy in symptomatic liver disease. Although all three salts - acetate, sulphate and gluconate are effective, acetate salts are preferred due to lesser incidence of gastric side effects.<sup>116</sup> Adults require 150 mg/day of elemental zinc in three divided doses while children and those under 50 kg, are given only 75 mg/day. Zinc should be taken on empty stomach to ensure better absorption. Besides clinical and biochemical improvement, treatment efficacy is determined by a 24-hour urinary copper excretion less

than 100 µg. Urinary excretion of zinc should be more than 2000 µg/day to ensure compliance and also to determine the quality of the zinc preparation used. Zinc is used as first line drug in pre-symptomatic patients or symptomatic patients with neurological WD and for long term maintenance therapy in others after optimal de-coppering with chelators.<sup>117-119</sup> In a recent study, hepatic treatment failure occurred more frequently on zinc therapy than chelator therapy. Patients who did not respond to zinc therapy showed hepatic improvement after reintroduction of a chelating agent. This suggests that chelating agents are better first line medications in symptomatic hepatic WD.

### ***Ammonium Tetrathiomolybdate***

Ammonium tetrathiomolybdate, originally used to treat copper poisoning in veterinary practice is a chelating drug with anti-angiogenic properties.<sup>120-121</sup> If ammonium tetrathiomolybdate, is taken after meals, it binds to the copper in the food, thus preventing its absorption. If taken on empty stomach, it is absorbed into the blood and forms a complex with circulating copper preventing cellular uptake; leading to its excretion in urine. The dosage used is 20 mg three times a day with meals and 20 mg three times a day in between the meals. Due to its aggressive chelating effects, the reported side effects of ammonium tetrathiomolybdate includes paradoxical worsening, bone marrow suppression, and hepatotoxicity. However, it has been stated that the potential neurological deterioration and side effects are lesser in comparison to trientine.<sup>122,123</sup> Currently ammonium tetrathiomolybdate is not available in India in many countries. Ongoing phase -2 multicenter trials with a more stable form of a chelator for copper (bis-choline tetrathiomolybdate (WTX101) report that 57% patients show improvement in liver function test and 72% show improvement in free copper levels by week 24 of therapy.<sup>124</sup> Further data on the use of this drug for various phenotypes of WD is awaited

## **Treatment phases**

### ***Initial phase***

This phase aims to reduce the body copper levels to sub-toxic threshold. The choice is between chelators (DP or trientine) alone, zinc alone, or a combination of both. There are no randomised controlled trials comparing the three and each centre uses a protocol based on their experience and patient compliance.<sup>2,125</sup>

DP has traditionally been the drug of choice unless the patients develop intolerance, in which case trientine is the preferred drug. In a study comparing DP versus zinc sulfate as first-

line therapy for Neuro-WD, the neurological worsening on DP vs zinc within 180 days of starting the drug was 35% vs 19%.<sup>126</sup> Authors concluded that DP and zinc sulfate were both effective in the majority of Neuro-WD patients. Neither therapy appears to be clearly superior. Therefore, zinc may be considered a reasonable alternative to DP as a first-line therapy. Centres using both chelator and Zinc together believe that DP has most beneficial effect in the early de-coppering phase, while zinc is inexpensive and assists by preventing copper absorption. There is insufficient data to prove the superiority of combination therapy but if it used, the two drugs must be given 6 hours apart to prevent chelation of zinc by the chelator.

### ***Maintenance Therapy***

This is life long and prevents copper re-accumulation after the patient has been effectively de-coppered. Zinc, in view of its good efficacy, low cost and toxicity, is the drug of choice. DP in low dose is an alternative but patients should be monitored for side effects.

### **Pre-symptomatic patients**

An asymptomatic sibling diagnosed to have WD by biochemical or genetic testing should be treated to prevent symptomatic disease. Zinc is the drug of choice. If a neonate is diagnosed to have WD based on genetic testing, it is unclear when treatment should be started. Because of the risk of body copper depletion, it should probably not be started in the first year.<sup>3</sup> It may be suggested that since clinical presentation is rare below 3 years, zinc therapy could start at the age of 2 years.

### **Wilson's disease presenting as acute liver failure (ALF) with / without encephalopathy**

Patients with encephalopathy should be considered for urgent liver transplantation. DP/trientine with or without zinc may be started as an ad hoc measure, but survival is unlikely without transplantation. In patients without encephalopathy, the decision on transplantation should be individualized after discussion with the relatives. While delay carries the risk of sudden deterioration, encephalopathy and death, unnecessary surgery may mean removing a native liver that may have recovered with medical treatment. The fulminant presentation with intense jaundice, hemolytic crisis and rapid deterioration of hepatic encephalopathy rarely survive without a transplant. Rapid removal of free copper through Molecular Absorption Recirculating System (MARS) or Total Plasma Exchange (TPE) can benefit patients with fulminant presentation. TPE efficiently removes both ceruloplasmin-bound and albumin-bound copper and the fresh frozen plasma used for exchange can be helpful in treating the associated coagulopathy. MARS is also effective<sup>127,128</sup> but more expensive and less widely available than TPE.<sup>129</sup>

These modalities are seen as bridge to LT, rather than a definitive treatment option.<sup>130</sup> In a recent study, 9 of 10 patients of WD, apheresis was a successful bridge to transplantation.

### **Liver transplantation**

#### ***Liver transplantation for Hepatic Wilson's disease***

Durand et al. in 2001 reported that early administration of DP may avoid LT for a vast majority of patients (90%) presenting with fulminant WD without HE at admission.<sup>102</sup> In 2006 Nazer's score<sup>131</sup> (serum bilirubin, INR and serum albumin) was modified to add two parameters (AST and WBC count) to the score and renamed it as New Wilson's Index (NWI). A NWI score  $\geq 11$  (table 5) was associated with non-survival without liver transplantation.<sup>77</sup> In a study from South India,<sup>132</sup> NWI and PELD/MELD were found to have modest accuracy in predicting outcome in WD. The authors derived a formula from regression analysis based on hepatic encephalopathy and bilirubin to predict the outcome in a fulminant presentation WD. Similarly, Fischer et al<sup>133</sup> identified 3 of the 6 patients who had NWI scores predictive of death and of these 3, 2 survived without a transplant. They cautioned about the scores not being accurate and this subgroup needing further study.

A recently published multi-centric cohort (comprising of 75 adults and 56 children) from France,<sup>134</sup> the Kaplan Mier survival analysis revealed good 5-year survival post LT (86% - 96%) depending on whether transplants were performed before or after the year 2000. This reflects improvements in the selection of patients and peri-operative management.

#### ***Liver transplantation for Neurological WD***

The indications for LT in neurologically affected patients is controversial. In a French study,<sup>134</sup> only 6% received a LT for a purely neurological indication and these patients were significantly worse after LT when compared to hepatic patients. All three patients with severe axial Parkinson's syndrome died (from infection) with a functional graft but without any neurological improvement. Although there is evidence that mild to moderate neurological involvement may improve after LT,<sup>135</sup> neuropsychiatric disease is a predictor for poor outcome after LT.<sup>136</sup> In a retrospective Italian study, neurological symptoms significantly improved after liver transplantation, but the survival of patients with hepatic and neuropsychiatric disease was significantly lower than those with liver disease alone.<sup>135</sup>

Sepsis was the main cause of death since neurological sequelae led to lack of ambulation and bedridden patients. LT as a treatment option for those with only neuropsychiatric disease is not recommended.

#### ***Liver grafts from heterozygous donors (siblings/parents)***

These are safe for both recipient and donor and disease recurrence risk is almost non-existent. In developing countries like India where deceased donor liver transplant program is still evolving and most transplants are from heterozygous related donors

### **Treatment monitoring**

Patients should be regularly monitored for ensuring compliance, efficacy of therapy and early recognition of side effects. Effective de-coppering (table 6) is assessed on 24-hour urine copper and serum free copper value. Serum free copper is calculated by the formula (serum copper - 3 x serum ceruloplasmin). If serum ceruloplasmin is not measured by the enzymatic method then this free copper calculation is not reliable. A 24-hour urine protein is estimated for renal toxicity of DP. This is initially done after a month, then 3 monthly and subsequently 6-12 monthly.

In hepatic WD, clinical improvement is characterised by decreasing jaundice, ascites, and portal hypertension. Complete blood counts and liver function tests are performed initially after a week, then at 2 and 4 weeks followed by 3 months, 6 months and then yearly. Child Pugh score (based on serum bilirubin, prothrombin time, serum albumin, presence of ascites and encephalopathy) and MELD score (based on bilirubin, creatinine and INR) should be documented in those with severe liver disease.

In neurological WD, symptoms on sequential evaluation remain the most critical outcome of therapeutic benefits. Scales are essential to objectively quantify the severity of the disease and its impact on patient's lifestyle. Over the years, of the many rating scales that have been used, the Global Assessment Scale for Wilson's Disease<sup>137</sup> (GAS for WD) is the preferred scale since it assesses the neuropsychiatric, hepatic and osseomuscular changes and their impact on quality of life over the observation period. (Table 7)

### **Long term Outcomes of Wilson's Disease**

Wilson's disease is well recognized as one of the treatable genetic disorders and early recognition and institution of therapy holds the key for good outcome. The response to therapy is dependent on various factors including drug compliance, duration/severity of symptoms at the time of institution of therapy. The Eurowilson Consortium in 2013,<sup>139</sup> analysed the outcomes of patients on oral chelators in treatment of WD with a follow up of 5 to 30 years. They noted that in 9/326 on Penicillamine, and in 3/141 on trientine, liver transplantation was needed. Overall improvement on therapy was 90% in

hepatic presentation and >55% with neurological presentation. In studies that have observed the long term outcomes in large cohorts of patients, good improvement (including near normal quality of life, improvement in symptoms or stabilization) was noted in 85-93.5%<sup>42,104,140,141</sup>. Most patients tend to have improvements up to 18-30 months after initiating therapy with good compliance, following which there is a plateau effect. Poor prognostic factors of patients of clinically severe neurological WD, included strong family history and severe MRI brain changes<sup>142</sup>. Despite severe neurological involvement, 50% of patients have good clinical improvement while on treatment. Non-responders to therapy show progressive, MRI worsening over time.

### **Symptomatic management of Neuro Wilson's Disease**

**Dystonia:** In addition to the de-coppering therapy, patients with moderate to significant dystonia need medications like trihexyphenidyl, tizanidine, baclofen, clonazepam, tetrabenzine for symptomatic treatment.<sup>143</sup> Patients who have do not respond to the above or have focal dystonia, botulinum toxin injections is the best option.<sup>142</sup> Deep brain stimulation and lesionectomies of (globus pallidus interna has been performed with variable benefits, in those with refractory symptoms. Plasamphoresis has been tried, for patients with refractory generalized dystonia, but is not part of standard protocol.

**Tremors:** Mild tremors do not require any specific interventions or only simple physical therapies. In patients who have tremors affecting activities of daily living, drugs including propranolol, clonazepam, anticholinergics, topiramate, primidone have been used.<sup>143</sup> Pallidotomy or deep brain stimulation can be considered in resistant tremors, with some beneficial effects.

**Parkinsonism:** Levodopa should be tried in all patients with parkinsonism, which might relieve symptoms. Other drugs of benefit include dopamine agonists, monoamine oxidase inhibitors, and amantadine.<sup>143</sup>

**Seizures:** Occur in about 6-8% of patients with Wilson's disease, either at initial presentation or during the course of the disease. Therapy is on standard lines with anti-epileptic medications. In view of associated hepatic dysfunction, anti-epileptics with first pass metabolism in liver should be avoided.

**Psychiatric Symptoms:** While mild symptoms improve gradually with de-coppering, those with severe symptoms require medical interventions. These include simple behavioural modifications to selective serotonin reuptake inhibitors, tricyclic antidepressants, serotonin-norepinephrine reuptake inhibitor and atypical antipsychotics (typical antipsychotics can precipitate extrapyramidal symptoms). Patients with aggressive manic symptoms or significant psychosis have been treated with electroconvulsive

therapies. There are no good randomized trials that have evaluated the efficacy of physical therapy. However isolated case reports and small case series suggest that physical therapy can be useful in cases with dystonia where stretching and splints can help prevent contractures and parkinsonism where gait and balance techniques can be benefit. There have been no randomized trials on the benefits of speech therapy for dysarthria. Neuromuscular electrical stimulation has been attempted in to improve dysphagia<sup>145</sup> and it is standard practice to use a percutaneous endoscopic gastrostomy (PEG) in patients where swallowing is markedly affected to improve nutrition until the patient is able to swallow well.

### **Dietary Copper in the management of Wilson's Disease**

No prospective studies are available to define a cut-off level of copper in the diet of a patient with Wilson's disease. Less than 1-2 mg/day of copper is widely acceptable and not specific for body weight or age (pediatric or adult). The AASLD and EASL recommend avoiding foods with high concentration of copper in the first year of treatment.<sup>1,2</sup> The rationale is justified as the body should not be overloaded with high dietary Cu during the critical first year of systemic chelation. However this opinion is based on two case reports of Wilson's disease on vegetarian diet and bioavailability of copper in healthy subjects with dietary changes.<sup>9</sup> The evidence to restrict Cu is weak. In developing countries, this recommendation has practical difficulties especially in vegetarians, since efforts to restrict dietary Cu to <2 mg/day results in protein intake being invariably reduced to 1-1.5 g/kg/d. This may be insufficient for a cirrhotic or advanced liver disease. Well-designed studies will be required to address this issue. It may be practical to restrict only very high copper containing foods like nuts, soy, liver and chocolates especially in the first year of chelation. Dairy, vegetables and fruits can be allowed unrestricted. Spices need not be restricted as effective intake is <5 g/d. Contrary to the wide belief, mushroom, animal meat, fish and poultry do not contain high copper. Table 8 shows food copper content in commonly used food items. The Cu content of foods is enhanced by drying, roasting, pickling, canning and adding preservatives, while boiling, milling, polishing and refining reduces the Cu content.<sup>146</sup> If Cu pipe tubing systems is used for domestic supply, it is important to check whether the water supplied is soft or hard. Soft water is acidic and tends to solubilise the copper from the pipelines with considerable "leaching effect" in stored samples. Hard water is neutral to alkaline and copper is unaffected. Drinking water should ideally contain <0.1 part per million (0.1mg/L) of Cu. It is important to let the first 1-2 litres of water be flushed away before using for drinking purposes. While there is no evidence that boiling, simple filtration or reverse osmosis reduces the copper content, ion exchange methods used in

ultrafiltration may be effective. Bottled mineral water is estimated to contain  $<0.05\text{mg/L Cu}$ .<sup>147</sup> However further studies are required to confirm the same. Studies on Indian childhood cirrhosis have shown that copper content of boiled and 6 hour stored buffalo milk ( $98.4\text{-}98.8\text{ }\mu\text{mol/L}$ ) and water ( $5.1\text{-}5.4$ ) in copper and brass vessels are higher than glass and aluminium vessels (milk:  $1.8\text{-}2.0$  and water  $0.9\text{-}1.1\text{ }\mu\text{mol/L}$ ).<sup>148</sup>

### **Additional treatment**

All patients need to be vaccinated for Hepatitis B and for Hepatitis A if they have not been previously exposed. Patients need to be managed for their complications of cirrhosis as per standard medical treatment. Alcohol and non steroidal anti-inflammatory drugs need to be avoided.

### **Young women with Wilson's disease**

Menstrual disturbances such as amenorrhea or oligomenorrhea are possibly related to disturbed hormonal activity. Impairment in conception and recurrent miscarriages in pregnancy have been reported. Women with undiagnosed and untreated WD have an increased rate of spontaneous abortions compared to women on treatment. (OR):  $2.853$  [95% CI:  $1.634\text{-}4.982$ ].<sup>149</sup> Proposed mechanisms include ovarian dysfunction due to decreased levels of estradiol with increased testosterone concentrations secondary to liver dysfunction and placental abnormalities like copper deposition. Higher spontaneous abortion noted in patients presented with neurologic manifestation compared with asymptomatic or hepatic or mixed manifestations (OR:  $2.335$ , 95% confidence interval [CI]:  $1.323\text{-}4.118$ ).<sup>150</sup> Patients on D-penicillamine and zinc showed lower spontaneous abortion rates compared to trientine (10% vs. 17% vs. 28%); but it was not statistically significant.<sup>150</sup>

### **Counselling the family**

Counselling also should address the risks of medication and risks of uncontrolled disease. Patients planning for pregnancy should have their copper status optimized before pregnancy. Also, the risk of 0.5% of the child being affected needs to be explained.

### **Maternal and pregnancy outcomes**

Several small case series and case reports in the last decade demonstrated a favourable outcome of pregnancies in patients treated with DP. Recent case reports with trientine and a case series of 29 WD patients exclusively treated with zinc showed uneventful maternal and pregnancy outcomes.<sup>150,151</sup> A

recently published largest study of 282 pregnancies in 136 women with WD showed a favourable course of pregnancies in vast majority of treated patients.<sup>152</sup> In this study, presentation was 54% hepatic, 32% neurologic and 9% were neurohepatic. Forty-one percent were on D-penicillamine, 13% trientine, 7% zinc, 3% combination treatment and no therapy in 31%. Ninety- three percent had unchanged maternal outcome, 6% hepatic deterioration and 1% neurological deterioration. Seventy-two percent had healthy babies and 26% had spontaneous abortion. Abortion rates were 20-29% in all presentations but 40% with neurological. Abortions were least with zinc (10%) and highest if undiagnosed without therapy (41%) or paused therapy (36%) Monitoring of liver functions and neurologic symptoms throughout pregnancy is of utmost importance. The study reported slight alteration of biochemical values in 6% of cases, which subsided after delivery.<sup>152</sup> Treatment interruption during pregnancy has been reported to result in acute liver failure and appearance of neurologic symptoms. Safety of chelating agents in pregnancy has not been well established. Teratogenicity with high dose DP has been reported in animal studies and there are isolated case reports of connective tissue defects in humans.<sup>153, 154</sup> Over zealous chelation and maternal copper deficiency can also result in impaired fetal development. Recent studies showed relatively low rate of birth defects (3%) with DP.<sup>152, 155</sup> and the authors concluded that the overall benefits of continuing treatment outweigh the risks of treatment withdrawal. To avoid overchelation, the dose of DP and trientine may be reduced by 25-50% while zinc can be continued in the same dose.<sup>152,156</sup> Patients whose diagnosis was made only during pregnancy should be started on standard dose of chelating agents considering the high copper burden. In women who are well controlled on chelation there is a suggestion that switching to zinc prior to conception decreases the risk of miscarriage or teratogenicity. Available data supports continuation of the previous chelator. Wound healing after Caesarean section or episiotomy may be delayed and therefore the dose of DP should be reduced toward the later weeks of pregnancy. After delivery, the dose of chelating agents should be increased to pre-pregnancy levels. Breast-feeding is not recommended in women on DP since it is excreted into breast milk and might be potentially harmful to the infant. There is scant data on safety of trientine and zinc in breast feeding. Detailed studies regarding contraception have not been performed so far. Spermicidal agents, barrier methods and progesterone only preparations are advisable for contraception in patients with WD. Estrogen containing pills may interfere with biliary copper excretion and some of the intrauterine devices contain copper.

### **Newer therapies**

Studies in murine models recently reveal that genetic WD mice carrying a corrective adenoviral vector containing human ATP7B DNA can bring long-term metabolic control and correction of copper metabolism.<sup>157</sup> Significant improvement in liver injury (biochemical and histological), ceruloplasmin levels, urinary copper excretion, hepatic copper load and increase in hepatic ATP7B expression by immunostaining has been documented. Cell therapy to repopulate the liver with healthy hepatocytes including the reconstitution of bile canalicular network has been tried.<sup>158</sup>

## **CONSENSUS STATEMENTS ON TREATMENT OF WILSON'S DISEASE**

### **Drug therapy**

- 1. A chelating agent should be used for symptomatic patients with hepatic Wilson's disease. D-penicillamine is preferred due to easy availability, cost and efficacy. (Strength-1, Level of evidence-B)**
- 2. Pre-symptomatic patients / those with hepatic disease on maintenance therapy / symptomatic patients with neuro- Wilson's disease can be treated with zinc or chelating agent (Strength-1, Level of evidence -A)**
- 3. Lifelong treatment is necessary unless the patient has had a liver transplant (Strength-1, Level of evidence -A)**
- 4. MARS and Total plasma exchange can be offered to patients with acute liver failure especially in presence of encephalopathy and is a good bridge therapy to LT, should the patient not show prompt recovery. (Strength 1 and Level of evidence B)**
- 5. D-penicillamine is an effective chelator although associated with several side effects. (Strength 1, Level of evidence A)**

### **Liver Transplantation**

- 1. Liver transplantation is indicated in patients with fulminant presentation with hepatic encephalopathy or hemolytic crises (Strength 1, Level of evidence B)**
- 2. New Wilson's Index has been used as a predictor of LT (for both acute or chronic presentation) Rising bilirubin, advanced hepatic encephalopathy and acute hemolysis have been suggested as better predictors for LT and need validation. (Strength 1, Level of evidence B)**

3. *Living donor liver transplant from heterozygous sibling is effective and safe for both donor and recipient (strength 1, Level of evidence B)*
4. *Liver transplantation is not indicated for in isolated severe neurological WD. When the liver is also diseased, the decision should be individualized, since significant neurological disease is a predictor of poor outcome. (Strength 1 and Level of evidence B)*

#### **Monitoring on treatment**

1. *Careful clinical monitoring is necessary to determine benefit and adverse effects of the drugs. Complete blood counts, urine analyses, liver function tests, 24 hour urinary copper and protein, serum free copper should be monitored frequently in the initial phase of therapy and at least 6-12 monthly thereafter. KF ring should be evaluated annually. (Strength-1, Level of evidence -B)*
2. *GAS for WD is a comprehensive reliable and valid scale. It should be used to objectively assess the disability and to check the response to treatment on follow up especially in patients with Neuro-Wilson's disease. (Strength 1, Level of evidence -B).*

#### **Drug therapy for neurological symptoms**

1. *Dystonia not responding to decoppering therapy anticholinergics and baclofen can be used. For those with refractory focal dystonia or residual dystonia, botulinum toxin injections can be considered (Strength 2, Level of evidence C).*
2. *Primidone and propranolol can be used in patients with residual postural and action tremors, levodopa in parkinsonism and tetrabenzine in hyperkinesias (Strength 2, Level of evidence C).*
3. *Physical therapy and speech therapy have some role to play in rehabilitation of patients with neurological Wilson's disease. (Strength 2, Level of evidence C).*
4. *In patients with severe dysphagia, PEG tubes are a temporary option till the dysphagia improves with chelation therapy. (Strength 2, Level of evidence C)*

#### **Maternal and pregnancy outcomes**

1. *Pregnancy in patients with WD is safe and most patients have successful outcome. [Strength I, Level of evidence B]*
2. *Patients with adequate copper control have better chances of successful pregnancies than untreated WD patients. Copper status of the patient should be optimized prior to pregnancy. [Strength I, Level of evidence B]*

1           **3. Treatment should be continued with previous chelation medication during pregnancy with**  
2           **dose reduction of chelators in later part of pregnancy. [Strength 2, Level of evidence B].**

3           **4. The risk of birth defects in WD is generally low, which also applies for patients treated with**  
4           **chelating agents. [Strength 1, Level of evidence B]**

5           **Newer Therapies**

6           **Newer therapies include hepatocyte transplant, stem cell transplant and gene therapy, which**  
7           **attempt to restore hepatobiliary copper excretion. However, these seem to be still in experimental**  
8           **phase. (Strength 2, Level of evidence C)**

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ACCEPTED MANUSCRIPT

Figures titles and legends where relevant

Figure 1: Circulation of copper in the body

Figure 2: Golden brown Kayser-Fleisher (KF) ring

Figure 3: Vacuous smile in Wilson's disease

Figure 4: MRI changes in Wilson's disease

Legend: A. Flair axial brain MRI showing hyperintensities in bilateral putamen and thalami with hypointensity of globus pallidi in a patient with neurological manifestations; B) T1W axial brain MRI showing hyperintensity of bilateral globus pallidi in a patient with hepatic form of WD; C) Flair axial brain MRI showing hyperintensities in dorsal midbrain and subcortical white matter signal changes in temporal region in a patient with neurological form of WD; D) T2W axial MRI brain depicting 'face of giant panda'; E,F: Central pontine myelinolysis-like Pontine signals changes in WD in flair axial brain MRI.

Figure 5: Elastosis perforans serpiginosa secondary to long term d-penicillamine use

Legend: Multiple annular plaques seen over bilateral forearms with Hyperpigmented papules arranged at the periphery and few papules showing central small keratotic plugging

**Table 1. Clinical manifestations of Wilson's disease**

|                  |                                                                                                                                                                                                                                                        |
|------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Hepatic          | Asymptomatic hepatomegaly, persistently elevated transaminases, Acute hepatitis, Chronic hepatitis, Cirrhosis (compensated and decompensated), Acute liver failure, Acute on chronic liver failure, Fatty liver, Isolated splenomegaly, Cholelithiasis |
| Neuropsychiatric | Tremors, Dystonia, Parkinsonism, Choreoathetosis, Seizures, Dysarthria, Drooling, Clumsiness, Incoordination, Gait disturbance, Behavioural changes, Deteriorating school performance, Depression, Anxiety, Psychosis                                  |
| Osseomuscular    | Arthralgia, Arthritis, Fractures, osteoporosis, osteomalacia, chondromalacia                                                                                                                                                                           |
| Hematological    | Hemolytic anemia, Thrombocytopenia, Pancytopenia, Coagulopathy                                                                                                                                                                                         |
| Ocular           | Kayser-Fleischer rings, Sunflower cataracts                                                                                                                                                                                                            |
| Renal            | Renal stones, Renal tubular acidosis, Fanconi syndrome                                                                                                                                                                                                 |

**Table 2: Studies with prevalence of various clinical manifestations of Wilson's disease**

| Study                                    | Hepatic     | Neuro-psychiatric                                                           | Pre-symptomatic | Other                       |
|------------------------------------------|-------------|-----------------------------------------------------------------------------|-----------------|-----------------------------|
| Lee BH, et al<br>(n=245) <sup>69</sup>   | 134 (54.8%) | 55 (22.4%)                                                                  | 55 (22.4%)      | 1 (0.4%)<br>[Osseomuscular] |
| Taly AB, et al<br>(n=282) <sup>42</sup>  | 42 (15%)    | 219 (77.6%)<br>Neuro 195<br>Hepato-neuro 10,<br>psychiatric 7,<br>others 7] | 15 (5.4%)       | 6 (2%)<br>[Osseomuscular]   |
| Walshe JM.<br>(n=217) <sup>40</sup>      | 94 (43.1%)  | 97 (44%)                                                                    | 24 (11%)        | 2 (0.9%)<br>[Osseomuscular] |
| Cheng N, et al<br>(n=1222) <sup>70</sup> | 450 (37%)   | 592 (48.5%)                                                                 | 31 (2.5%)       | 149 (12%) [Neuro-visceral]  |

**Table 3: Modified Leipzig scoring system for diagnosis of Wilson's disease**

|                                                            |   |
|------------------------------------------------------------|---|
| KF rings                                                   |   |
| Present                                                    | 2 |
| Absent                                                     | 0 |
| Serum ceruloplasmin                                        |   |
| Normal (> 20 mg/dl )                                       | 0 |
| 0 – 5 mg/dl                                                | 3 |
| 6 -11 mg/dl                                                | 2 |
| 11-20 mg/dl                                                | 1 |
| 24 hour urinary copper (in the absence of acute hepatitis) |   |
| >100 mcg                                                   | 2 |
| 40-100                                                     | 1 |
| <40 mcg                                                    | 0 |
| Coombs-negative hemolytic anemia with liver disease        |   |
| Present                                                    | 1 |
| Absent                                                     | 0 |
| Mutational analysis                                        |   |
| On both chromosomes detected                               | 4 |
| On one chromosome detected                                 | 1 |
| No mutation detected/ test not done                        | 0 |
| Liver biopsy for histology S/O WD                          |   |
| Orcein or Rhodanine-positive granules                      | 1 |

|                               |   |
|-------------------------------|---|
| Neurobehavioral symptoms      | 2 |
| Typical features on MRI BRAIN | 1 |

|                                                                                                                            |   |
|----------------------------------------------------------------------------------------------------------------------------|---|
| Any one of the following<br>Family history or<br>Sibling death from liver disease/Neurological<br>disease suggestive of WD | 1 |
|----------------------------------------------------------------------------------------------------------------------------|---|

TOTAL SCORE

Evaluation

≥4 or more

Diagnosis established

3

Diagnosis possible, more tests needed

≤2

Diagnosis very unlikely

WD – Wilson's disease

**Table 4: Adverse Effects of Drugs used in treatment of Wilson's disease**

| Name of drug     | Side Effects                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| D-penicillamine- | <p><b>Early:</b> (1-3 weeks) Hypersensitivity</p> <p>Fever, cutaneous eruptions, lymphadenopathy, neutropenia, thrombocytopenia and proteinuria</p> <p><b>Late:</b> (3 weeks to 3 months) Hypersensitivity</p> <p>a) Renal: Lupus like syndrome (proteinuria, hematuria, positive ANA)</p> <p>b) Lung: Good pasture syndrome</p> <p>c) Bone Marrow: severe thrombocytopenia, total aplasia</p> <p>d) Skin: pemphigus, pemphigoid lesions involving skin, mouth, vagina, buccal ulcerations, aphthous stomatitis, hair loss</p> <p>e) Eye: optic neuritis.</p> <p><b>Very Late</b> (after 1 year )</p> <p>Nephrotoxicity, severe allergy on restarting drug, myasthenia gravis, polymyositis (&lt;1%), loss of taste, immunoglobulin A depression, retinitis, hepatotoxicity (transaminitis), copper depletion leading to neutropenia, sideroblasticanemia and hemosiderosis</p> <p><b>Direct Dose dependent:</b></p> <p>a) Pyridoxine deficiency</p> <p>b) Skin: Elastosisperforansserpentina, lichen planus, progeria like skin changes, d-penicillaminodermopathy a brownish discolouration of skin secondary to trauma related subcutaneous bleed</p> <p>c) Mammary hypertrophy</p> <p><b>Neurological deterioration:</b> Incidence ranges from 10-50%</p> |

|                                                     |                                                                                                                                                                                                                                                                                                                                                               |
|-----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                     |                                                                                                                                                                                                                                                                                                                                                               |
| Triethylenetetramine<br>hydrochloride/<br>Trientene | Few side effects<br>No hypersensitivity described<br>Fixed drug eruption reported in one patient.<br>Chelates Iron: should not be co-administered with Iron<br>Bone marrow depression<br>Sideroblasticanemia: reversible (Iron and copper Deficiency )<br>Hemorrhagic gastritis, Loss of taste and skin rash<br><br>Neurological deterioration is less common |

ANA: anti-nuclear antibody

**Table 5: New Wilson's index for predicting survival** <sup>75</sup>

| Score | Bilirubin<br>(mg/dl) | INR       | AST(IU/L) | WCC<br>(10 <sup>9</sup> /L) |
|-------|----------------------|-----------|-----------|-----------------------------|
| 0     | 0 – 5.8              | 0 – 1.29  | 0 – 100   | 0 – 6.7                     |
| 1     | 5.9 – 8.7            | 1.3 – 1.6 | 101 – 150 | 6.8 – 8.3                   |
| 2     | 8.8 – 11.6           | 1.7 – 1.9 | 151 – 200 | 8.4 – 10.3                  |
| 3     | 11.7 – 17.5          | 2.0 – 2.4 | 201 – 300 | 10.4 – 15.3                 |
| 4     | >17.6                | >2.5      | >300      | >15.4                       |

**Table 6: Interpretation of tests used in monitoring drug treatment of Wilson's disease**

|                                    | Zinc                                                          | D-penicillamine / Trientine                               |
|------------------------------------|---------------------------------------------------------------|-----------------------------------------------------------|
| Initial treatment                  | U Cu 100-500 µg/d<br>S free Cu > 25 µg/dL<br>U Zn > 2000 µg/d | U Cu >500µg/d<br>S free Cu > 25 µg /dL                    |
| Good control<br>(Maintenance)      | U Cu <75µg/d<br>S free Cu 10-15 µg /dL                        | U Cu 200-500 µg/d<br>S free Cu 10-15µg /dL                |
| Non-compliance/<br>Inadequate dose | U Zn <2000µg/d<br>S free Cu >15µg /dL                         | U Cu < 200 µg/d<br>U Cu > 500 µg/d<br>S free Cu >15µg /dL |
| Over-treatment                     | U Cu < 25 µg/d<br>S. free Cu < 5 µg /dL                       | U Cu < 200 µg/d<br>S. free Cu < 5 µg /dL                  |

To document therapeutic efficiency, urinary copper excretion after 2 days of D-d-penicillamine cessation should be < 50µg in 24 hours. If more, suggest poor compliance<sup>136</sup>

U Cu = 24 hour urinary copper, U Zn = 24 hour urinary zinc, S. free Cu = Serum free copper, DP = D penicillamine

**Table 7: Global Assessment Scale for Wilson's disease**

| Tier                                                                                                                           | Areas of Assessment                                                                                                                                                                                                                                                                                                                                                          |                                                                           |
|--------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Tier 1 Domains<br>Scored from 0-5 in each domain based on impact on activities of daily living                                 | Domain                                                                                                                                                                                                                                                                                                                                                                       |                                                                           |
|                                                                                                                                | Liver                                                                                                                                                                                                                                                                                                                                                                        | Clinical, biochemical and abdominal ultrasound evidence for liver disease |
|                                                                                                                                | Cognition and Behaviour                                                                                                                                                                                                                                                                                                                                                      | Intellectual decline, depression and psychosis                            |
|                                                                                                                                | Motor                                                                                                                                                                                                                                                                                                                                                                        | Motor impairment on examination                                           |
|                                                                                                                                | Osseomuscular                                                                                                                                                                                                                                                                                                                                                                | Joint or muscle involvement by clinical examination and radiographs       |
| Tier 2<br>Items 1-13 scored from 0-4 based on clinical severity                                                                | <ol style="list-style-type: none"> <li>1. Wilson's facies</li> <li>2. Scholastic performance</li> <li>3. Depression</li> <li>4. Psychosis</li> <li>5. Dystonia</li> <li>6. Tremor</li> <li>7. Chorea</li> <li>8. Parkinsonism</li> <li>9. Speech</li> <li>10. Swallowing</li> <li>11. Salivation</li> <li>12. Posture and gait</li> <li>13. Kayser Fleischer ring</li> </ol> |                                                                           |
| Tier 2 Item 14 is scored on presence or absence of uncommon manifestations (each scored as 1 point with a maximum of 4 points. | Emotional lability<br>Seizures over preceding 1 month<br>Myoclonus<br>Stereotypy<br>Tics<br>Pyramidal signs<br>Eye movement abnormalities                                                                                                                                                                                                                                    |                                                                           |

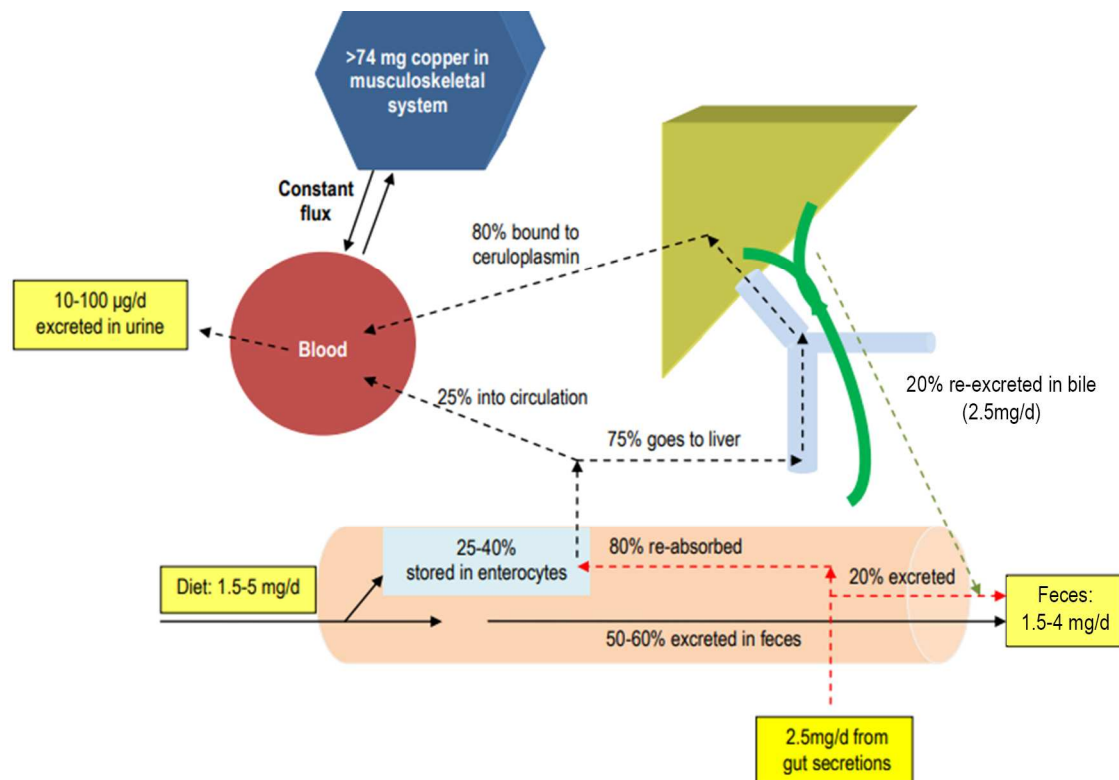
\* In the liver domain, the scores range from no liver disease ever (L0) through active liver disease (L2), compensated (L3), decompensated liver disease (L4) to potentially life threatening disease (L5)

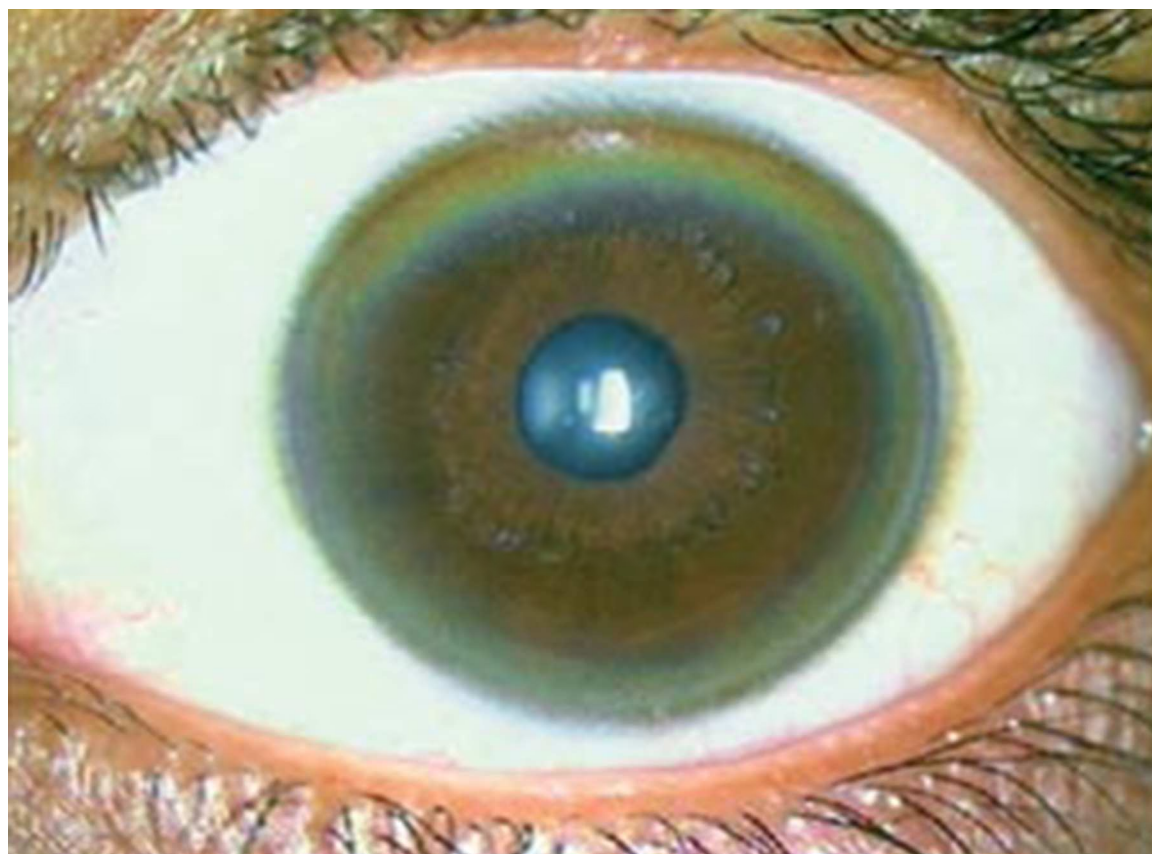
Greater weightage is given to Wilson's facies and K-F rings that are characteristic features of Wilsons disease.

**Table 8: The copper content (mg) in daily dietary items per 100 g edible portion  
(Adapted from Indian Food Composition Table, National Institute of Nutrition  
2017)**

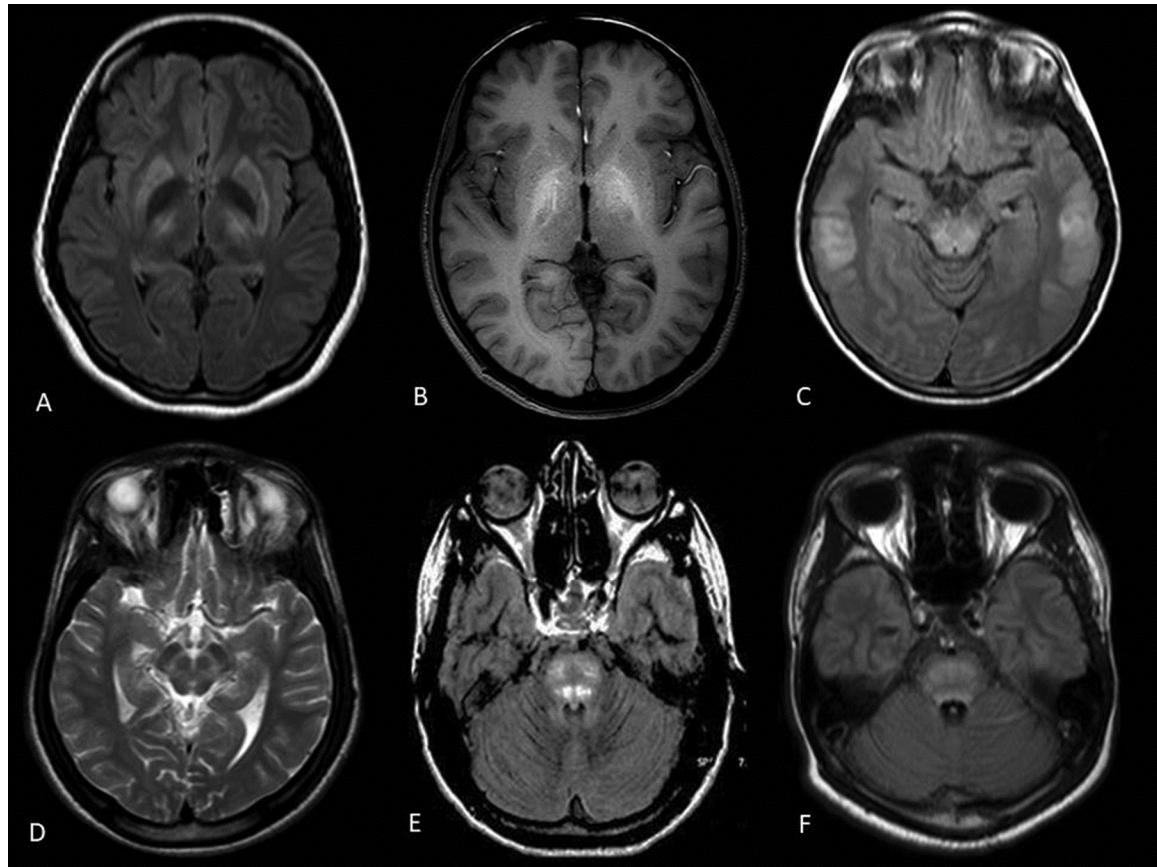
| <b>Low copper food items</b><br>(copper content <1mg/100g edible portion)                                                                                                                                         | <b>High copper food items</b><br>(copper content >1mg/100g edible portion) |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Rice (whole, puff, flakes) (0.23-0.27)                                                                                                                                                                            | Red gram (1.14)                                                            |
| Wheat (whole grain, flour, semolina, vermicelli and noodles) (0.17-0.48)                                                                                                                                          | Soyabean (1.29)                                                            |
| Maize and products (0.11-0.45)                                                                                                                                                                                    | Lotus stem, water chestnut (1.2-1.3)                                       |
| Barley and millet (0.43-0.67)                                                                                                                                                                                     | All nuts (1.1-2.2)                                                         |
| All legumes except red gram (0.6-0.97)                                                                                                                                                                            | Cumin, coriander, black pepper and mace (1.1-1.6)                          |
| All vegetables (0.1-0.4)                                                                                                                                                                                          | Liver (6.0)                                                                |
| Mushroom (0.09)                                                                                                                                                                                                   | Oyster (3.4)                                                               |
| All fruits (0.1-0.6)                                                                                                                                                                                              | Duck meat (1.0)                                                            |
| All spices (except cumin, coriander, black pepper and mace) (0.1-0.6)                                                                                                                                             | Cocoa (3.8)                                                                |
| Fish, prawns, chicken, red meat (0.1-0.5)                                                                                                                                                                         |                                                                            |
| Egg (0.07)                                                                                                                                                                                                        |                                                                            |
| Milk and dairy products (0.03-0.1)                                                                                                                                                                                |                                                                            |
| Coffee; tea (0.2-0.5)                                                                                                                                                                                             |                                                                            |
| Jaggery (0.03)                                                                                                                                                                                                    |                                                                            |
| The authors cannot verify the Cu content of the following food items: gram flour, barley, oats, protein energy powder, soy milk, soy products, sauces, cheese and table salt. Gram flour and barley are likely to |                                                                            |

contain higher Cu content from their original pulses as the process requires drying and roasting. Most protein energy powders are derived from soy sources. All soy products, sauces and cheese undergo fermentation and contain preservatives thereby increasing the Cu content.











ACCEPTED