RESEARCH ARTICLE

Demographic Characteristics of Iraqi Patients with Wilson Disease and Clinical Hepatic Variations among Different Treatment Protocols

Fadwa G. Hameed,[1]* Mohamed Mahmood,[2] Inam Sameh,[3] Hala Sameh[4]

[1]*Department of Pharmacy, Al-Kitab University, Kirkuk, Iraq.*
[2]*Department of Clinical Pharmacy, College of Pharmacy, University of Al-Mustansiriyah, Baghdad, Iraq.*
[3]*Department of Pharmacology and Toxicology, College of Pharmacy, University of Al-Mustansiriyah, Baghdad, Iraq.*
[4]*Department of Pediatrics, College of Medicine, Al-Nahrain University, Baghdad, Iraq.*

Received: 09th March, 2021; Revised: 26th April, 2021; Accepted: 17th May, 2021; Available Online: 25th June, 2021

ABSTRACT

The Wilson disease (WD) is inherited but possibly curable disorder of copper metabolism designated by the pathological aggregation of copper. The cause of WD is mutations in ATP7B, which encodes a transmembrane copper- transporting ATPase, taking it to impaired copper homeostasis and copper overload in various organs. The severity and type of symptoms may vary the clinical approach of WD, but progressive liver disease is standard. Clinical presentation of WD can vary widely; therefore, diagnosis is not always straightforward. Wilson disease is not just a disease of children and young adults but may be available at any age. The main features of WD are liver disease and cirrhosis, neuropsychiatric disturbances, Kayser–Fleischer rings, and acute episodes of hemolysis, often in association with acute renal failure. Diagnosis is challenging in children and adults presenting with active liver disease. Innovative screening techniques may increase ascertainment. Regularly, the researchers are developing new diagnosis methodologies and treatment of WD.

Keywords: ATP7B, Copper, D-penicillamine, Hepatic, Neurological, Psychiatric, Trientine, Wilson disease, Wilson ATPase, Zinc salts.

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.2.40

How to cite this article: Hameed FG, Mahmood M, Sameh I, Sameh H. Demographic Characteristics of Iraqi Patients with Wilson Disease and Clinical Hepatic Variations among Different Treatment Protocols. International Journal of Drug Delivery Technology. 2021;11(2):462-468.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

WD, also known as hepatolenticular degeneration, is an autosomal recessive disease because of mutations in the ATP7B gene on chromosome 13 that results in the congregation of toxic concentrations of copper in the liver, central nervous system, eye, kidney, skeletal muscle, bone, synovia, and heart.[1,2] WD is a fatal disease unless excessive tissue copper is removed with the help of copper chelating agents and/or zinc inhibition of intestinal absorption of dietary copper.[3] WD is an autosomal, recessive genetic disease caused by homozygous or compounds heterozygous mutations in the ATP7B gene located on chromosome 13q14.3 that encodes a transmembrane copper-transporting P-type ATPase(ATP7B; Wilson ATPase).[4,5] Most patients are compound heterozygotes. Mutations of ATP7B impair transport of excess copper into bile for elimination in feces and impede transport of copper from intracellular chaperone proteins into apoceruloplasmin for the synthesis of ceruloplasmin in hepatocytes. It is now standard to sequence all 21 ATP7B exons, and more than 600 pathogenic variants in ATP7B have been identified. The gene frequency is estimated to be 1 in 90–150, making it more common than perceived earlier. Single-nucleotide missense and nonsense mutations are general, followed by insertion/deletion mutations and rare splice site mutations. Hence, various mutations may result in clinical manifestations of copper overload. Variations in presenting features exist, however, even among individuals having identical mutations. The Histidine to glutamine substitution at amino acid 1069 (H1069Q) is the most common mutation in WD, and its population allelic frequency is 10%–40% (30%–70% in Caucasians).[5] WD occurs in children and adults worldwide in all races and ethnicities.[1,2,6] Most patients present between the ages of 5 and 35, and WD can present from 8 months of early age to e ninth decade of life. Genetic studies have revised the estimated prevalence to142 per 106, far exceeding the prior estimate of 30 per 106. The higher estimate indicates a discrepancy between the number of patients diagnosed with WD and the numbers predicted by genetic studies. Reduced penetrance of some ATPB7 mutations may explain some of the

**Author for Correspondence: fadwa.g.hameed@uoalkitab.edu.iq*

discrepancies. However, it is likely that failure to diagnose WD patients with only mild or atypical features also contributes to the discrepancy. Geographic clustering with consanguinity has resulted in even higher prevalences in Romania (885 per 106) and Sardinia (370 per 106 births).[6] Copper is a trace mineral important to health because of its obligatory role in copper-dependent enzymes, such as ceruloplasmin, tyrosinase, lysyl oxidase, dopamine-β-monooxygenase, and peptidylglycine amidating monooxygenase.[1,2,6] It is crucial that clinicians remain aware that WD can cause dysfunction in multiple organ systems and that the presenting clinical or laboratory features of WD are often initially subtle and may mimic alternative diagnoses.[1,2] Primary hepatic presentations are most common between the ages of 5 and 40 but may occur sooner or later. Currently, presentation with asymptomatic elevations of aminotransferase enzymes is increasingly common. Mild hepatomegaly may be present. Patients may also have advanced liver fibrosis or cirrhosis, complications of portal hypertension, hepatocellular carcinoma, or acute liver failure. A retrospective analysis reported that 62% of 229 patients with overt hepatic manifestations had cirrhosis when diagnosed.[9] In addition, up to 11% of asymptomatic patients also had cirrhosis at diagnosis.[10] Hemolysis contributes to the magnitude of bilirubin elevation in patients with acute liver failure, and an acute episode may be the presenting feature in approximately 10%. Low-grade hemolysis may occur, even with quiescent liver disease, and some patients have intermittent worsening of hemolysis, resulting in episodic jaundice. Patients with hepatic disease may also have neuropsychiatric signs or symptoms. Among 14 patients with neurologic symptoms over age 40, liver biopsies showed advanced fibrosis in 10 (71%).[11] Some other study on 34 patients with neurologic WD reported cirrhosis in 14 (41%),[10] corroborating a prior study showing cirrhosis in 11 of 23 patients (48%) who presented with neurologic WD.[12]

MATERIALS AND METHODS

A total of 42 Iraqi patients diagnosed with Wilson disease (20 males and 22 females) with age range between 10–20 years attended the Rare Disease clinic of the Al-Imaamin AL-Kadhmian medical city Hospital, Baghdad, Iraq. The diagnosis of WD was established by clinical features, low serum ceruloplasmin, and copper and increased 24 hours urinary copper excretion, Liver function tests.

Inclusion Criteria

- Patients age from 10-20 years old.
- Asymptomatic patients with WD with hepatic involvement diagnosed by screening method.
- Patients referred for the unexplained elevation of liver enzymes and $^{+}$ve Leipzig scale score.
- Patients with a positive family history of Wilson disease.

Exclusion Criteria

- Patient >20 years old.
- Patients with other comorbidities.
- Pregnancy and breastfeeding.
- Patients were allergic to any of the study medications.

The present study is an interventional prospective randomized controlled,open-label designed to evaluate the role of different diagnostic tools and the effect of various treatment protocols in Iraqi patients with Wilson disease. This study was conducted between September 2019 to April 2020.

Study Groups

The eligible 42 patients were allocated into three groups:

Group (1); including 23 asymptomatic Iraqi patients with WD and hepatic involvement diagnosed by screening method treated with Dietary Supplement: Zinc acetate(100-150 mg/ d) in 2-3 divided dose for 90 days. Blood tests was performed (before and after the treatment): ALT, AST, ALP, Ca., S. Albumine, Total Albumin, PT, IRN, S. Copper, S. Ceruplasmine, 24 hour urine copper.

Group (2); included 10 Iraqi Patients referred for the unexplained elevation of liver enzymes and (+ve) Leipzig scale score treated with d-PCA (20 mg/kg/d for Ped., 750–1500 mg for an adult) for 90 days. Blood tests performed (before and after the treatment): ALT, AST, ALP, s.Albumine, Total Albumin, PT, IRN, S. Copper, S. Ceruplasmine, 24 hour urine copper.

Group (3); included 9 Iraqi patients referred for unexplained elevation of liver enzymes and (+ve) Leipzig scale score treated with Trientine (20 mg/kg/d for Ped., 1500–2500 mg for an adult) for 90 days. Blood tests will be performed(before and after the treatment): ALT, AST, ALP, s.Albumine, Total Albumin, PT/IRN, S. Copper, S. Ceruplasmine, 24 hour urine copper.

RESULTS

The baseline characteristics of the study patients were presented in Table 1. The gender distribution of the study groups (female: male) ratio was (52.38%:47.62%) demonstrated as follows: Group 1 (50% vs 50%), group 2 (55.56% vs 44.44%) and group 3 (43.5% vs 56.5%) respectively.The largest number of patients (23%) in group 3 were aged (6–20) years, in group 2 (9%) of patients were aged range (7–19) years, in group 1 (10%) of patients were aged (9–14) years. Most patients in this study have a positive family history for WD represents (7.14%). Meanwhile, patients with negative family history represent (92.85%) of all patients. Positive surgical history was reported in (11.21%)group 1 patients, (22%) in group 2 patients and (30.43%) in group 3 patients. The duration of symptoms of < 1 years was reported (54.76%), duration of symptoms between (1-2) years was reported (45.23%). There was no statistically significant difference in the mean values of gender, age, family history, the surgical history between the study group's patients ($P > 0.05$), while the duration of symptom (< 1 year) shows a significant p-value **(**0.0236).

Hepatic variation among the study groups

Table 2 and Figures 1, 2, and 3 demonstrates the hepatic parameters used in the study groups (D-penicillamine, Trinetin, and Zinc Acetate). Liver function tests were evaluated among the patient groups (D-penicillamine, Trinetin, and Zinc Acetate), ALT and AST show no significant P-value while

Table 1: Demographic Data and Disease Characteristics of Patients with Wilson's disease

Variables	*D-penicillamine (Group-1)*	*Trinetin (Group-2)*	*Zinc Acetate (Group-3)*	*p value*
Gender [a]:				
Male	5 (50%)	5 (55.56%)	10 (43.5%)	
Female	5 (50%)	4 (44.44%)	13 (56.5%)	0.81 NS
Total	10 (100%)	9 (100%)	23 (100%)	
Age [b]:	11.5±1.95	14.78±4.86	14.22±4.01	0.12 NS
Family history [b]:	3 (30%)	4 (44.44%)	7 (30%)	
Negative				0.72 NS
Positive	7 (70%)	5 (55.56%)	16 (70%)	
surgical history b:	2 (20%)	4 (44.44%)	4 (17%)	
Positive				0.25 NS
Negative	8 (80%)	5 (55.56%)	19 (83%)	
Symptoms duration b:	4 (40%)	3 (33%)	18 (78%)	
< 1 year				0.02 *
1-2 years	6 (60%)	6 (67%)	5 (22%)	

Data presented as Mean ±SD, (n) is the number of patients and (%) is a percentage.
(a): Pearson chi-square test, (b): FEPT,
NS: Non significant ($P > 0.05$).

Table 2: Diagnostic hepatic tests for Wilson disease used in the study groups

Variables	*D-penicillamine*	*Trinetin*	*Zinc Acetate*	*P.value*
ALT.	62.55 ± 14.57	76.72 ± 26.99	59.86 ± 17.98	0.0989 NS
AST.	55.03 ± 12.11	65.37 ± 17.13	56.6 ± 23.93	0.4812 NS
ALP.	180.6 ± 44.79	379.1 ± 137.6	196.9 ± 121.5	0.0003**
T.Bilirubin	0.956 ± 0.6465	2.289 ± 1.533	1.101 ± 0.8048	0.0064**
Albumin	0.218 ± 0.1001	0.2156 ± 0.1979	0.327 ± 0.1119	0.0360*

NS = Non-significant, * significant at p value ≤ 0.05, ** significant at p value ≤ 0.01

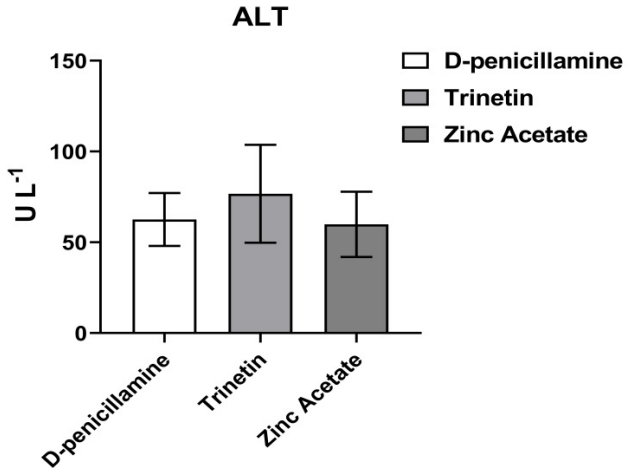

Figure 1: ALT. variation levels among the three groups of treatment.

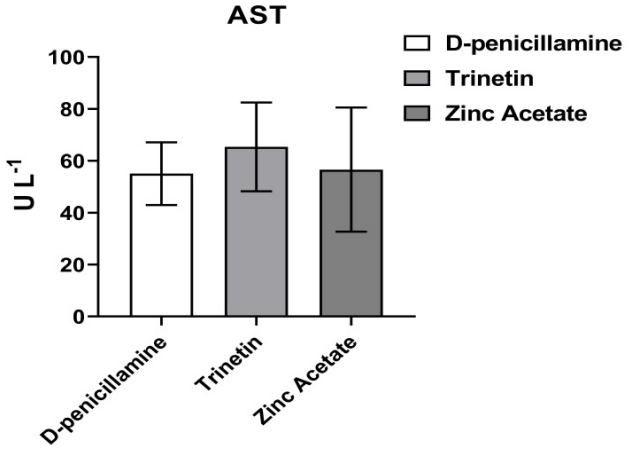

Figure 2: AST. Variation levels among the three groups of treatment.

ALP and Total Bilirubin shows significant P.value (≤ 0.05). Albumin shows a significant P-value (≤ 0.01).

DISCUSSION

The present study and other studies revealed that WD occurs in children and adults worldwide in all races and Ethnicities.[2,13] Our study observed gender differences with a slight predominance towards male patients in our population.[14] Age variation in our study was between 6–20 years old, and While most patients present between the ages of 5 and 35, Genetic studies have revised the estimated prevalence to142 per 106, far exceeding the prior estimate of 30 per 106. The higher estimate indicates a discrepancy between the number of patients diagnosed with WD and the number predicted by genetic studies. Reduced penetrance of some ATPB7 mutations

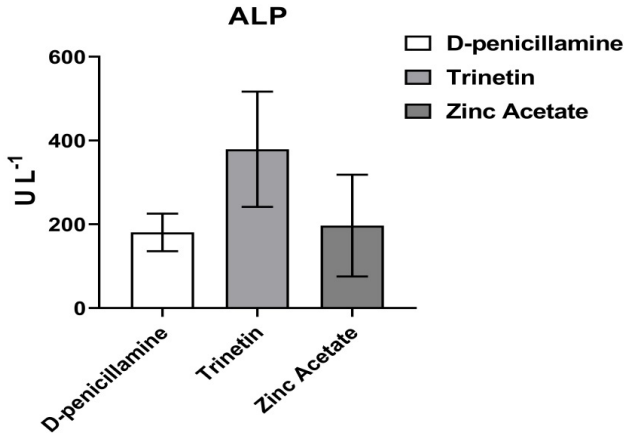

Figure 3: ALP. variations levels among the study groups.

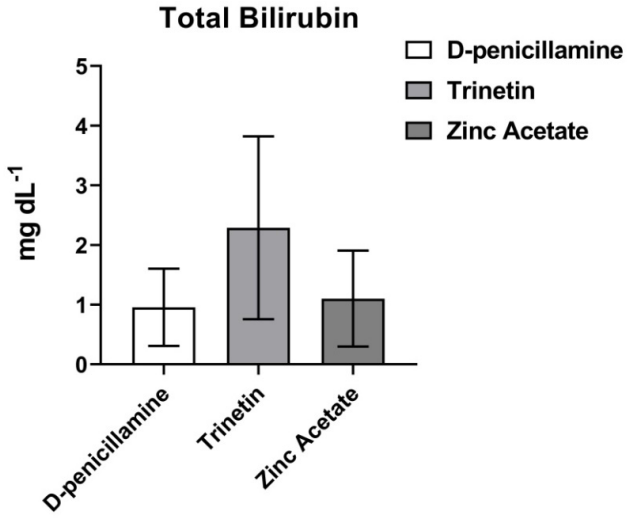

Figure 4: Total Bilirubin variations levels among the study groups.

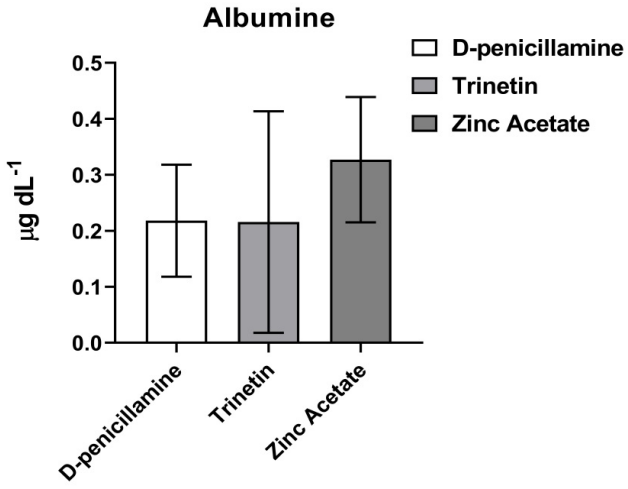

Figure 5: Albumin variations levels among the study groups.

may explain some of the discrepancies. However, it is likely that failure to diagnose WD patients with only mild or atypical features also contributes to the discrepancy.[13] The presentation of WD varies according to age and gender. Merle *et al*.[15] reported the mean age of symptom onset as 15.5 years for those with hepatic and 20.2 years with neurologic involvement. Litwin *et al*.[16] reported gender differences in 627 consecutive WD cases. Neurologic presentations were more common at diagnosis in men than women (60% vs. 39%), and hepatic presentations were more common in women than men (58% vs. 41%). These patterns may help in our understanding of the pathophysiological basis for phenotypic variation but should not influence decisions to investigate WD. While most cases present between the age of 5 and 35 years old,[17] the range is wide for both hepatic and neurologic presentations.[18]

In the present study, a positive family history of WD was found in approximately (64%) of patients. WD is highly recommended to perform familial screening; American Association for Study of Liver Diseases (AASLD) and European Association for Study of Liver (EASL) recommend screening first-degree relatives of the pro band, suggesting siblings or offspring only. It is usually considered that WD not only occurs in siblings (25%) and the offspring (0.5%), but it also occurs in the previous generation (0.5%), although rarely reported.[19] This was convenient with the Brunet *et al*.[20] reported a 43-year-old asymptomatic father diagnosed with WD after his daughter was diagnosed with a typical WD. Similarly, a 14-year-old girl was diagnosed of having WD in Korea.[12]

Further study for her family members revealed that her father, a paternal uncle, and a sister were compound heterozygous WD patients. Even though this risk is low, screening parents and the children of a proband is justified given the potential devastating process of WD. Parents are often considered heterozygous. Although most WD patients were younger than 40 years, several researchers reported old patients diagnosed with WD in their early 80s. It is reported that a 43-year-old asymptomatic father screened and confirmed WD after his daughter was diagnosed with a typical WD.[19,22] In an old study, a 41-year-old mother of a proband was also diagnosed as a pre-symptomatic patient with WD through whole ATP7B gene sequencing.[23] Considering incidence equaling to the previous generation, screening for the next generation is also necessary. A study in two Sardinian families found two atypical patients with WD in offsprings by genetic testing. Other similar findings have been reported.[20,21]

Clinicians must be aware that WD can cause dysfunction in multiple organ systems and that the presenting clinical or laboratory features of WD are often initially subtle and may mimic alternative diagnoses. Astute clinicians should keep WD in mind when evaluating various signs, symptoms, and laboratory abnormalities and be prepared to initiate diagnostic testing whenever suspicious of that diagnosis. Majority of patients in the current study presented with a duration of symptoms of less than one year, while patients with a duration of symptoms between (1–2) years reported less. The clinical features may vary from an asymptomatic

state with biochemical abnormalities, hepatic diseases, neurologic deficits, and psychiatric disorders.[24,25] Diagnosis is established by a clinical scoring system proposed by "8th International Meeting on Wilson Disease and Menkes Disease, Leipzig (2001)" presenting clinical and laboratory parameters lacking diagnostic accuracy.[26] In our study, Liver function tests were evaluated among the patient groups (D-penicillamine, Trinetin, and Zinc Acetate), ALT, and AST show no significant difference among the study groups while ALP, Albumin, and Total Bilirubin shows significant differences (Figures 3-5). The aim of treating WD creates a negative copper balance within the body. This can be achieved by increasing the amount of copper lost in urine by using chelators such as D-penicillamine or trientine and/or decreasing copper absorption from the digestive tract by using zinc salts.[27-29]. Even without extensive clinical prospective studies to assess its efficacy over more than last 60 years, D-penicillamine remains the most frequently used drug worldwide to treat WD.[28,30] However, more recent knowledge of WD pathogenesis, new therapeutic possibilities, and clinical observations over more extended periods of follow-up reveal that caution is warranted when using this drug.[28-31] During appropriate WD treatment with D-penicillamine at an adequate dosage and with good adherence, one must monitor clinical markers of liver disease, including jaundice, ascites, and coagulopathy, along with serum aminotransferases and measures of synthetic hepatic function for the recovery of liver function, particularly during the first year of treatment.[27] Non-adherence to treatment, as is often the case in chronic disorders, leads to disease progression and liver failure; the occurrence or recurrence of neurological symptoms is common.[28,32] Serum alkaline phosphatase levels are disproportionately low, while total bilirubin is disproportionately high due to the concomitant rise of indirect bilirubin from copper-induced hemolysis. Transaminases may be only mildly elevated.

An acute presentation may be observed in patients previously treated for Wilson's disease. However, in whom medication is stopped.[33] As an oral chelator, D-penicillamine is specified as a first-line drug for use in symptomatic WD patients according to recommendations published by the European Association for the Study of Liver (EASL) (Grade II-1, B, 1) and the American Association for the Study of Liver Diseases (Class I, Level B). However, discussions regarding the superiority of trientine versus D-penicillamine (in terms of adverse events), as well as the possibility of substituting zinc salts for D-penicillamine, continue. Despite these debates, there have been no prospective randomized controlled studies to compare the safety and efficacy of the different drugs used to treat WD. Instead, most available data are derived from retrospective studies and expert opinion. Other than confirming the use of D-penicillamine in WD, the current EASL guidelines discuss the role of zinc salts as a first-line treatment in neurological WD.[28]

Some studies investigated adherence and efficacy of trientine therapy in children with WD.[34-37] In one of the two most extensive series, 10 of 15 patients were started on a trientine treatment regime.[37] After confirmation of adequate chelation, as determined by urinary copper levels, trientine therapy was accompanied by zinc acetate before discontinuation of trientine and conversion to zinc monotherapy. During a 18 months follow-up period, liver function improved steadily. In this cohort, the authors observed no adverse events. They concluded that trientine (6zinc) therapy is well tolerated and effective for children with WD. In another retrospective study, 16 children were identified as having received trientine at some point during their treatment. In this cohort, trientine was mainly used as second-line option secondary to severe adverse events with D-penicillamine. Notably, trientine was as efficacious as D-penicillamine to achieve normalization of liver function tests and cessation of symptoms. In line with other studies, the adverse reactions were less frequent in the trientine group, prompting the authors to consider trientine as first-line treatment strategy.[36] An attractive feature of zinc therapy for WD is that mechanistically it is more specific in removing copper than either D-penicillamine or trientine. Reported clinical experience is the main source of data for determining zinc therapeutics in WD.[40] There are almost no randomized-controlled clinical trials.[38,41] A valuable resource is the systematic review [181], performed by Houwen's group in Utrecht as part of the EuroWilson FP6 project: papers in English, French, or German from 1966 up to January 2008 in Medline plus all years in the EMBASE and Cochrane databases were included, and animal studies were excluded. The objective was to identify the best initial monotherapy for WD: only 13 papers met their criteria for evaluation. Data for zinc therapy include only zinc sulfate and zinc acetate as actual interventions. The authors of this review concluded that zinc monotherapy is probably preferable for silent WD (asymptomatic/ pre-symptomatic patients) and patients presenting with predominantly neurological manifestations, but it might not be the best choice as primary therapy for those with hepatic WD.

Direct comparisons (two) of zinc and D-penicillamine were considered for systematic review, and it was found that zinc was also valuable in the treatment of WD. Zinc is also considered better than D-penicillamine as it has lesser adverse effects than D-penicillamine.[38,40] An sooner and non-critical, comprehensive review of zinc acetate define it as having appreciative efficacy and safety.[182] A recent retrospective study of 143 newly diagnosed WD patients found that zinc was similar D-penicillamine in terms of efficacy and tolerability.[42] The other recent retrospective review of pre-symptomatic patients, identified by first-degree relative screening, treated with either D-penicillamine or zinc showed that over a median follow-up of 12 years (ranging 3–52 years), 63% of patients remained symptom-free.[43] Clinical diseases were developed in non-compliant patients. It was observed that during the time, the patients were inclined to select zinc primarily due to its safety profile. The current treatment strategy is depending upon the child's clinical status and age. Concerning hepatic

WD, current expert opinion specifies oral chelators for clinically proven liver disease and zinc monotherapy for the asymptomatic child;[39] however, these recommendations are not appropriately rated. Keeping this approach is valuable, especially as it relates to zinc, under ongoing evaluation. The Zinc treatment on children with WD requires considerable individualization.

The Liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), typically normalize over the first 6–18 months of treatment. In patients, few tests with advanced liver disease may not normalize but should improve and stabilize. Persistently, abnormal liver enzymes (more than the twice upper limit of normal) may be markers of under-dosing, non-adherence, treatment failure, or secondary liver injury.[44] Dose adjustment, assessing adherence, or alternative therapy should be considered. Liver enzyme normalization reduces the risk of fibrosis growth.

Total bilirubin may be elevated with hemolysis, and enhanced direct bilirubin is a marker of liver dysfunction. Levels of serum albumin are expected to improve with therapy and nutrition. Although serum transaminases (ALT and AST) are referred to as liver tests, they are markers of liver injury.[45] In one retrospective study in Europe, the presence of liver tests (ALT and AST) that are twice the baseline value or more than that after treatment for a time (typically beyond 3–6 months) would be considered a possible treatment failure and dose changes or alteration in therapy considered.[46]

Albumin and INR are markers of the actual function of liver cells; however albumin is influenced by inflammation (it is a negative acute-phase marker), by nutritional state (low in malnourished states), and when there is loss of albumin from the kidney (proteinuria) or the gut (in protein-losing enteropathy). Therefore following the international normalized prothrombin time (PT) or INR reflects liver function more directly, with some exceptions.[47]

CONCLUSION

Clinically, Wilson's Disease is found from early childhood till the ninth decade of life. Most of the patients have been found to be compound heterozygotes for mutations in the ATP7B gene on chromosome 13q14.3. Clinical presentations are found to vary a lot and hence it is advised to the clinicians to consider WD in the differential diagnosis of acute and chronic liver diseases, neuropsychiatric diseases, and hemolysis. The diagnosis methods include serum ceruloplasmin, neurologic exam, and slit-lamp examination for Kayser Fleischer rings. However, more testing for basal 24-hour urinary copper excretion, hepatic copper concentration, or sequencing of the ATP7B gene may also be considered. All family member who shares about 50 percent of their genes with a particular individual or in other words, first-degree relatives must be screened to identify presymptomatic WD. Copper stores that are toxic in nature, may be subjected to be removed with chelators or zinc, which reduce the absorption of copper in the Intestine. Patients with an acute liver failure (prominently with classic Wilsonian acute liver failure associated with profound Coombs negative intravascular hemolysis), severe decompensation of cirrhosis, or hepatocellular carcinoma, go through Liver transplantation. Also, patients having neurological diseases don't get a recommendation for liver transplantation. We can observe that the prognosis of clinically treated patients with primary stages of WD and their presymptomatic relatives is appreciable and excellent.

REFERENCES

1. Roberts EA, Schilsky ML.Diagnosis and treatment of Wilson disease: an update. Hepatology.2008;47:2089-2111.
2. Author links open overlay panelEuropean Association for the Study of the Liver .EASL Clinical Practice Guidelines:Wilson's disease. J Hepatol.2012;56:671-685.
3. Czlonkowska A, Litwin T. Wilson disease—currently used anticopper therapy. Handb Clin Neurol.2017;142:181-191.
4. Bull PC, Thomas GR, Rommens JM, Forbes JR, Cox DW. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. Nat Genet.1993;5:327-337.
5. Chang IJ, Hahn SH. The genetics of Wilson disease. Handb Clin Neurol. 2017;142:19-34.
6. Lo C, Bandmann O. Epidemiology and introduction to the clinical presentation of Wilson disease. Handb Clin Neurol.2017;142:7-17.
7. Scheiber IF, Bruha R, Dusek P. Pathogenesis of Wilson disease. Handb Clin Neurol.2017;142:43-55.
8. Boga S, Ala A, Schilsky ML. Hepatic features of Wilson disease. Handb Clin Neurol.2017;142:91-99.
9. Beinhardt S, Leiss W, Stattermayer AF, Graziadei I, Zoller H, Stauber R, *et al*. Long-term outcomes of patients with Wilson disease in a large Austrian cohort. Clin Gastroenterol Hepatol.2014;12:683-689.
10. Ferenci P, Steindl-Munda P, Vogel W, Jessner W, Gschwantler M, Stauber R, *et al*. Diagnostic value of quantitative hepatic copper determination in patients with Wilson's disease. Clin Gastroenterol Hepatol.2005;3:811-818.
11. Ferenci P, Czlonkowska A, Merle U, Ferenc S, Gromadzka G, Yurdaydin C, *et al*. Late-onset Wilson's disease. Gastroenterology.2007;132:1294-1298.
12. Przybylkowski A, Gromadzka G, Chabik G, Wierzchowska A, Litwin T, Czlonkowska A. Liver cirrhosis in patients newly diagnosed with neurological phenotype of Wilson's disease. Funct Neurol.2014;29:23-29.
13. Lo C, Bandmann O. Epidemiology and introduction to the clinical presentation of Wilson disease. Handb Clin Neurol.2017;142:7-17.
14. Tomasz Litwin, Grazyna Gromadzka, A Członkowska.Gender differences in Wilson's Disease. Journal of the Neurological Sciences. September 2011.
15. Merle U, Schaefer M, Ferenci P, *et al*.Clinical presentation, diagnosis and long-term outcome of Wilson's disease: a cohort study. Gut.2007;56:115-120.
16. Litwin T, Gromadzka G, Czlonkowska A. Gender differences in Wilson's disease. J Neurol Sci.2012;312:31-5.
17. Boga S, Ala A, Schilsky ML. Hepatic features of Wilson disease. Handb Clin Neurol.2017;142:91-99.
18. Samuel Shribman,ThomasT. Warner,and James S. Dooley. Clinical presentations of Wilson disease.Ann Transl Med. 2019 Apr; 7.
19. Huamei Li,Lifang Liu,Yun Li,Shendi He,Yujie Liu,Jinhong Li,Ran Tao,Wei Li,and Shiqiang Shang.Familial screening of children with Wilson disease.Necessity of screening in previous

generation and screening methods. Medicine (Baltimore).2018 Jul; 97(27)
20. Brunet AS, Marotte S, Guillaud O, *et al*. Familial screening in Wilson's disease: think at the previous generation. J Hepatol.2012;57:1394-1395
21. Roberts EA, Schilsky ML, AASLD Diagnosis and treatment of Wilson disease: an update. Hepatology.2008;47:1394-1395
22. Ala A, Borjigin J, Rochwarger A, *et al*.Wilson disease in septuagenarian siblings: raising the bar for diagnosis. Hepatology.2005;41:668-670.
23. Li H, Liu L, Li Y, *et al*.Familial screening of children with Wilson disease: Necessity of screening in previous generation and screening methods.Medicine (Baltimore).2018;97:e11405.
24. European Association for the Study of the Liver,"EASL clinical practice guideline: Wilson's disease," Journal of Hepatology,vol.56,2012:671-685.
25. A. Poujois and F. Woimant, "Wilson's disease," Clinics and Research in Hepatology and Gastroenterology, vol. 42, no. 6, pp. 512-520, 2018.
26. P. Ferenci, K. Caca, G. Loudianos *et al*., "Diagnosis and phenotypic classification of Wilson disease," Liver International, vol. 23, no. 3, pp. 139-142, 2003.
27. Brewer GJ. The modern treatment of Wilson's disease. J Gastrointest Dig Syst 2015;5:312.
28. European Association For The Study of The Liver Disease. EASL clinical practice guidelines:Wilson's disease. J Hepatol 2012;56:671-685.
29. Weiss KH, Stremmel W. Clinical considerations for an effective medical therapy in Wilson's disease. Ann NY Acad Sci 2014;1315:81-85.
30. Weiss KH, Thurik F, Gotthardt DN, Scha¨fer M, Teufel U, Wiegand F, *et al*. Efficacy and safety of oral chelators in treatment of patients with Wilson's disease. Clin Gastroenterol Hepatol 2013;11:1028-1035.
31. Weiss KH, Gothardt DN, Klemm D, Merle U, Ferenci-Foerster D, Schaefer M, *et al*. Zinc mono therapy is not as effective as chelating agents intreatment of Wilson disease. Gastroenterology 2011;140:1189-1198.
32. Dziezyc K, Karli´nski M, Litwin T, Członkowska A. Compliant treatment with anti-copper agents prevents clinically overt Wilson's disease in pre-symptomatic patients. Eur J Neurol 2014;21:332-337.
33. Walshe JM.The liver in Wilson's disease. In: Schiff L, Schiff ER. (eds) Diseases of the liver. 6th ed. Philadelphia, PA: J B Lippincott, 1987, pp.1037-1050.
34. Wang LC, Wang JD, Tsai CR, Cheng SB, Lin CC. Clinical features and therapeutic response in Taiwanese children with Wilson's disease: 12 years of experience in a single center. Pediatr Neonatol 2010;51:124-129.
35. Wiggelinkhuizen M, Tilanus ME, Bollen CW, Houwen RH. Systematic review: clinical efficacy of chelator agents and zinc in the initial treatment of Wilson disease. Aliment Pharmacol Ther 2009;29:947-958.
36. Taylor RM, Chen Y, Dhawan A, EuroWilson Consortium. Triethylene tetramine dihydrochloride (trientine) in children with Wilson disease: experience at King's College Hospital and review of the literature. Eur J Pediatr 2009;168:1061-1068.
37. Arnon R, Calderon JF, Schilsky M, Emre S, Shneider BL. Wilson disease in children: serum aminotransferases and urinary copper on triethylene tetramine dihydrochloride (trientine) treatment. J Pediatr Gastroenterol Nutr 2007;44:596-602.
38. Czlonkowska A, Gajda J, Rodo M. Effects of long-term treatment in Wilson's disease with D-penicillamine and zinc sulphate. J Neurol1996;243:269-273.
39. Socha P, Janczyk W, Dhawan A, Baumann U, D'Antiga L, Tanner S, *et al*. Wilson's disease in children: a position paper by the Hepatology Committee of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr 2018;66:334-344.
40. Brewer GJ, Dick RD, Johnson VD, Brunberg JA, Kluin KJ, Fink JK. Treatment of Wilson's disease with zinc: XV. Long-term follow-up studies.J Lab Clin Med 1998;132:264-278.
41. Askari FK, Greenson J, Dick RD, Johnson VD, Brewer GJ. Treatment of Wilson's disease with zinc. XVIII. Initial treatment of the hepatic decompensation presentation with trientine and zinc. J Lab Clin Med 2003;142:385-390.
42. Anderson LA, Hakojarvi SL, Boudreaux SK. Zinc acetate treatment in Wilson's disease. Ann Pharmacother 1998;32:78-87.
43. Czlonkowska A, Litwin T, Karlinski M, Dziezyc K, Chabik G, Czerska M. D-penicillamine versus zinc sulfate as first-line therapy for Wilson'sdisease. Eur J Neurol 2014;21:599-606.
44. Iorio R, *et al*. Serum transaminases in children with Wilson's disease. J Pediatr Gastroenterol Nutr. 2004;39(4):331-336.
45. Roberts EA, Schilsky ML, American D, Association for Study of Liver. Diagnosis and treatment of Wilson disease: an update. Hepatology. 2008;47(6):2089-2111.
46. Weiss KH, *et al*. Zinc monotherapy is not as effective as chelating agents in treatment of Wilson disease. Gastroenterology.2011;140(4):1189-1198 e1.
47. Dhawan A, *et al*.Wilson's disease in children: 37-year experience and revised King's score for liver transplantation. Liver Transpl. 2005;11(4):441-448.