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Original Article

Clinical Characteristics, Treatment Effects and Risk Factors of Liver Cirrhosis in Patients with Wilson's Disease Hepatic Type

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Abstract

Background and Aims: Wilson's disease (WD) is a rare autosomal recessive genetic disorder that can be treated with medications. The lack of a single, specific diagnostic indicator leads to diagnostic difficulties, which may result in disease progression to cirrhosis and even liver cancer. Thus, this study aimed to analyze the clinical data, imaging, histopathological manifestations, genetic testing results, and treatment effects of patients with WD hepatic type, and to explore the factors related to WD cirrhosis. ***Methods:*** A single-center retrospective study was performed. 48 WD patients with a Leipzig score ≥ 4 were divided into a cirrhosis group and a non-cirrhosis group based on the presence of cirrhosis. Logistic regression analysis and odds ratios were used to describe the strength of association between risk factors and cirrhosis. The predictive value of the model for cirrhosis occurrence was evaluated by calculating the area under the receiver operating characteristic curve and the cutoff value. ***Results:*** All 48 patients diagnosed with WD had liver damage, with males accounting for 54.17%. The median age at diagnosis was 28 years (range: 10.25–40.5 years), and 39.58% of patients had cirrhosis. The most prevalent mutation was c.2333G>T (p.Arg778Leu), found in 41.30% (19/46) of cases. Imaging revealed fatty liver in 31.25% (15/48) of patients and "honeycomb-like" cirrhosis nodules in 73.68% (14/19). Compared with the non-cirrhosis group, the cirrhosis group had a higher positive rate for the Kayser-Fleischer (K-F) ring, older age at diagnosis, and higher levels of immunoglobulin G, but lower levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, white blood cells, and platelets ($p < 0.05$). Age at diagnosis (odds ratio = 1.072, 95% confidence interval = 1.007–1.142, $p = 0.03$) and the K-F ring (odds ratio = 18.657, 95% confidence interval = 1.451–239.924, $p = 0.025$) were independent risk factors for WD-related cirrhosis. The best values of area under the receiver operating characteristic curve for age at diagnosis combined with the K-F ring in predicting WD cirrhosis were 0.909. The average follow-up time for 33 patients was 48.6 months (range: 12–72 months). The biochemical recovery rate was over 60% after 12–72 months of treatment with zinc gluconate and/or penicillamine. ***Conclusions:*** Age at diagnosis, combined with the K-F ring, is a simple and effective risk factor for WD-related cirrhosis. Zinc gluconate and penicillamine are safe and effective treatments.

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Introduction

Wilson's disease (WD) is a genetic disorder inherited in an autosomal recessive manner, resulting from mutations in the ATP7B gene located on chromosome 13 (13q14.3). These mutations lead to a decrease in the copper-transporting P-type ATPase encoded by ATP7B, which influences the biliary excretion of copper and the synthesis of serum ceruloplasmin. This causes pathological copper accumulations in several organs, including the liver, brain, kidneys, bones, and cornea, eventually leading to clinical symptoms.[1,2] While the prevalence of WD is reported to range between 0.25 and 4.00 per 10,000 people, the potential incidence is much higher.[3,4] WD stands out as an uncommon hereditary liver disease that can be treated. Timely diagnosis and treatment are critical in preventing or slowing the progression of the disease to cirrhosis or, in extreme cases, hepatocellular carcinoma.

Current research suggests that the clinical characteristics and earliest symptoms of WD are primarily hepatic, followed

Keywords: Wilson's disease; K-F ring; Age at diagnosis; Liver cirrhosis; Zinc gluconate; Penicillamine.

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by neurological.[5] Wilson's disease can affect people of all ages, but most studies focus on children or adults,[6–10] with only a few addressing both. Atypical clinical presentations are particularly prominent in pediatric research, with hepatic symptoms frequently serving as the primary symptom. There is no clear relationship between genetics and phenotype. Wilson's disease in adulthood often presents as a mixed variety, with neurological symptoms predominating. Serum ceruloplasmin is the primary laboratory test for detecting this disease, with studies indicating a negative relationship between serum ceruloplasmin levels and clinical symptom severity.[11] However, several investigations have found no differences between clinical categories.[5] Genetic testing is also an important diagnostic methods. Studies have shown that the c.3207C>A (p.His1069Gln) mutation is relatively common in Europe.[11–13] The c.2333G>T (p.R778L) mutation is more common in Asia and may be associated with severe liver damage.[10,14,15] Previous research has mostly focused on the link between clinical subtypes and biochemical indicators, while the relevant risk factors for WD cirrhosis remain unclear.

As a result, this study aimed to analyze the clinical data, imaging, histopathological manifestations, genetic testing results, and treatment effects of patients with WD hepatic type, and to explore the factors related to WD cirrhosis.

Methods

Patients

Demographic characteristics of 77 patients with a suspected diagnosis of WD, admitted to the Second Hospital of Nanjing between June 2017 and June 2023, were retrospectively collected. In this study, data analysis was anonymous, and all participants provided informed consent. This study conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the Second Hospital of Nanjing (2024-LS-ky044; June 19, 2024).

Enrollment criteria

Inclusion criteria: WD was diagnosed in patients who fulfilled the following criteria: the application of the diagnostic criteria established at the 8th International Conference on WD held in Leipzig in 2001, namely, the Leipzig scoring system,[16] with a total score of ≥4 points.

Liver cirrhosis: The presence or absence of liver cirrhosis at presentation was determined based on clinical characteristics, imaging results (computed tomography or magnetic resonance imaging), and blood tests.[17]

Exclusion criteria: Patients who did not meet the diagnostic criteria or provided missing information were excluded. Other causes of liver injury were excluded, including HBV, HCV, and HIV infections, drug-induced hepatitis, autoimmune liver disease, fatty liver disease, malignant tumors, and pregnant women.

Laboratory methods

Laboratory tests: A blood routine was conducted using the BC-3000 blood cell analyzer from Shenzhen Maiduan Company. Biochemical indices were measured using the automatic biochemical analyzer (OLYMPUS AU2700). Serum ceruloplasmin levels were assessed using immunonephelometry with the Beckman Coulter Immage 800 and standard reagents (Beckman Coulter, Brea, CA, USA). Twenty-four-hour urinary copper content and serum copper levels were detected by graphite furnace atomic absorption spectrometry.[18]

Corneal Kayser-Fleischer ring

The Kayser-Fleischer (K-F) ring is a yellow-green or yellow-gray pigment ring on the corneal edge that can be seen with the naked eye under the side light of a flashlight. If it is not visible, an ocular slit lamp should be used to examine the corneal K-F ring.[19]

Liver biopsy and histopathology assessment

Some patients underwent an ultrasound-guided liver biopsy using a 16G needle and an automatic adjustable biopsy instrument from American Bard. The tissue collected must measure more than 2.0 cm in length and contain at least 11 portal regions. After fixation, embedding, and continuous slicing, liver tissue specimens underwent staining with hematoxylin and eosin (H&E), reticulofibrillar Masson stain, or special stains under certain conditions.[20] Two expert liver pathologists analyzed the liver samples blindly. Hepatic tissue inflammation and fibrosis were scored using the Scheuer scoring system, with F4 indicating cirrhosis.[21]

Genetic testing

After patients signed a written informed consent form, a volume of 3 mL of venous blood was collected. Genomic DNA was isolated, and the target sequence library was created using polymerase chain reaction. The library was measured with a fluorescence quantimeter, and its length was assessed with an Agilent 2100 Bioanalyzer; high-throughput sequencing was then performed, and mutation information and annotations were analyzed using GATK and ANNOVAR software.[22]

Treatment options and follow-up

Penicillamine (125 mg/tablet) was used alone, with the maximum daily dose determined by the patient's tolerance; penicillamine combined with zinc gluconate (70 mg/tablet, containing 10 mg of elemental zinc, dosage: 15 tablets/day for those over 15 years old, seven tablets/day for those aged five to fifteen years, and five tablets/day for those under five years old); dimercaptosuccinic acid (0.25 g/tablet, dosage: three to four tablets/day for adults, 10–20 mg/kg/day for children) was an alternative treatment for patients allergic to penicillamine.[23] During treatment, patients were monitored for adverse reactions such as fever, rash, leukopenia or neutrophil count, thrombocytopenia, joint pain, proteinuria, etc. Changes in blood routine, liver and kidney function, electrolytes, urine copper, and liver, gallbladder, and spleen B-ultrasound were also monitored.[18] Follow-up started from the time of diagnosis of WD, with patients receiving standardized treatment. The follow-up deadline was June 2024 to evaluate treatment effects and disease progression (new cirrhosis, ascites, esophageal and gastric varices and rupture bleeding, liver cancer, etc.).

Statistical analysis

Data were analyzed using SPSS 22 software. Normally distributed data were expressed as mean ± standard deviation (X±SD) and compared by t-test. Skewed distribution data were expressed as median (Q1, Q3) and compared by the Mann-Whitney U test. Categorical variables were reported as percentages, and between-group comparisons were performed with the χ^2 test or Fisher's exact test. Logistic regression analysis was used to estimate the univariate and multivariate effects of different risk factors on the development of liver cirrhosis. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to describe the strength of the association between the risk factors and cirrhosis. The receiver operating characteristic curve was plotted, and the

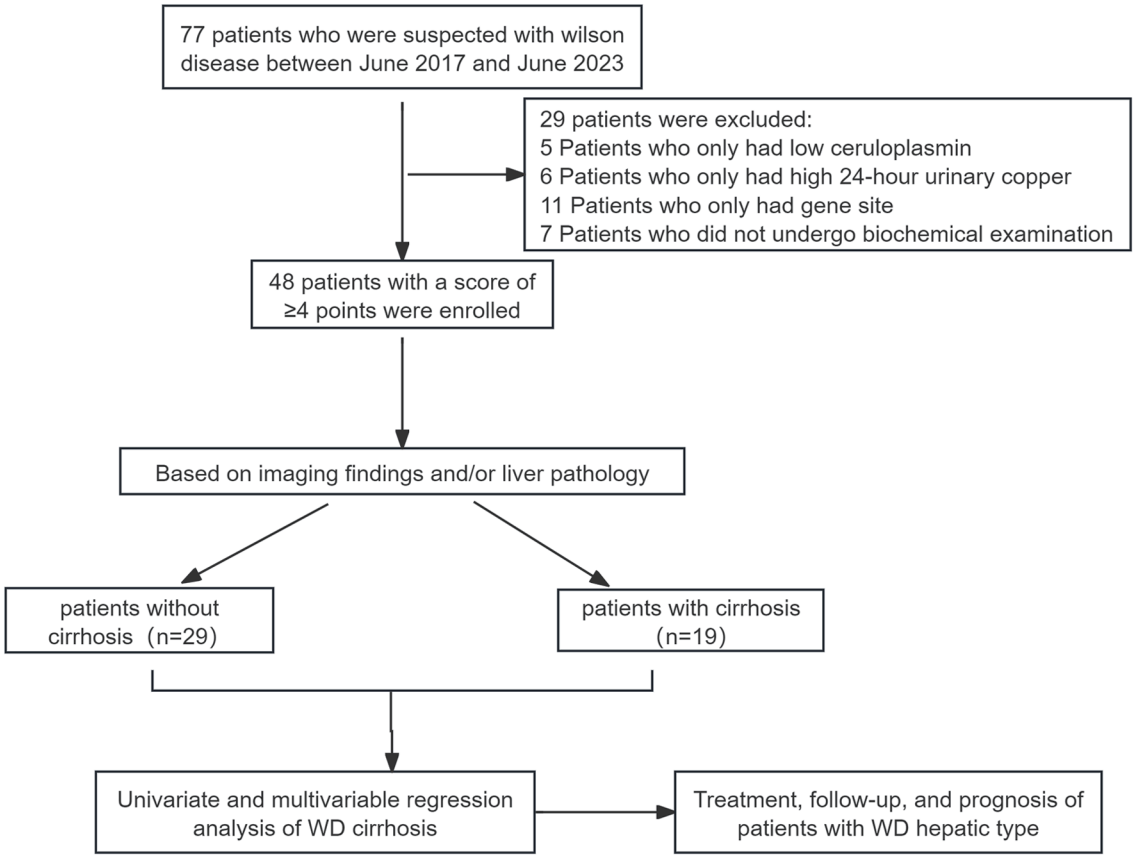

Fig. 1. Study design flowchart. WD, wilson's disease.

area under the receiver operating characteristic curve (AUC) and the cut-off value were calculated to evaluate the model's predictive value for cirrhosis occurrence. A significance level of $p < 0.05$ was considered statistically significant.

Results

Clinical baseline characteristics of WD of hepatic type

Initially, 77 patients were enrolled. Forty-eight were included due to a Leipzig score ≥ 4, while 29 were excluded because they had only one abnormal result in this study (Fig. 1). All of the patients had liver injury, three of whom had neurologic involvement, and one presented with hemolysis. There were 26 males (54.17%) and 22 females (45.83%). The median age at diagnosis was 28 (10.25, 40.5) years, with 58.33% (28/48) being ≥18 years old and 39.58% (19/48) having cirrhosis. The median serum copper level was 308.45 (187, 369.45) μg/L (reference range: Children ≤ 16y: 800.0–1,900.0 μg/L; Adults: Male > 16y: 700.0–1,400.0 μg/L; Female > 16y: 800.0–1,550.0 μg/L), while the median serum ceruloplasmin level was 84.0451 (47, 111.5) mg/L (reference range: 230–440 mg/L). The median 24-h urinary copper content was 236.9 (114.07, 516.93) μg/24 h (reference range: 15–60 μg/24 h).

Ninety-five point eighty-three percent (46/48) of patients underwent genetic testing. Two mutation sites were the most common (73.91%, 34/46), followed by one mutant site (21.74%, 10/46), with one patient having a homozygous mutation (c.2621C>T (p.Ala874Val)), and the rest being heterozygous. Three mutations were the least frequent (4.35%, 2/46). The most prevalent mutation was c.2333G>T (p.Arg778Leu) in 41.30% (19/46), followed by c.2975C>T (p.Pro992Leu) and c.2621C>T (p.Ala874Val), each in 15.22% (7/46), respectively. Serum ceruloplasmin, 24-h urinary copper, and the K-F ring all exceeded 4 points on the Leipzig scoring system in the two patients who did not undergo genetic testing.

A liver biopsy was performed on 12 patients. Inflammation score ≥ 2 was detected in 75.00% (9/12), and fibrosis score ≥ 2 was detected in 50.00% (6/12). Among these, steatosis accounted for 75% (9/12), with steatosis grade ≥ 1 in 88.89% (8/9). Seventy-five percent (9/12) had interface inflammation, 66.67% (6/9) had mild interface inflammation, 33.33% (3/9) had moderate interface inflammation, and 16.66% (2/12) had intrahepatic cholestasis in zone 3 (Fig. 2).

Imaging findings suggest that fatty liver accounted for 31.25% (15/48) of patients, cirrhosis in 39.58% (19/48), with 73.68% (14/19) of the cirrhotic nodules showing "honeycomb" changes. Fifty percent (24/48) of the patients had splenomegaly, 14.58% (7/48) had ascites, and 4.16% (2/48) had esophageal varices.

Comparison of clinical characteristics and laboratory data in the cirrhosis and non-cirrhosis groups

Compared to the non-cirrhosis group, the cirrhosis group had an older age at diagnosis ($p < 0.05$), higher levels of immunoglobulin G, and a higher positive rate of K-F ring (9/19,

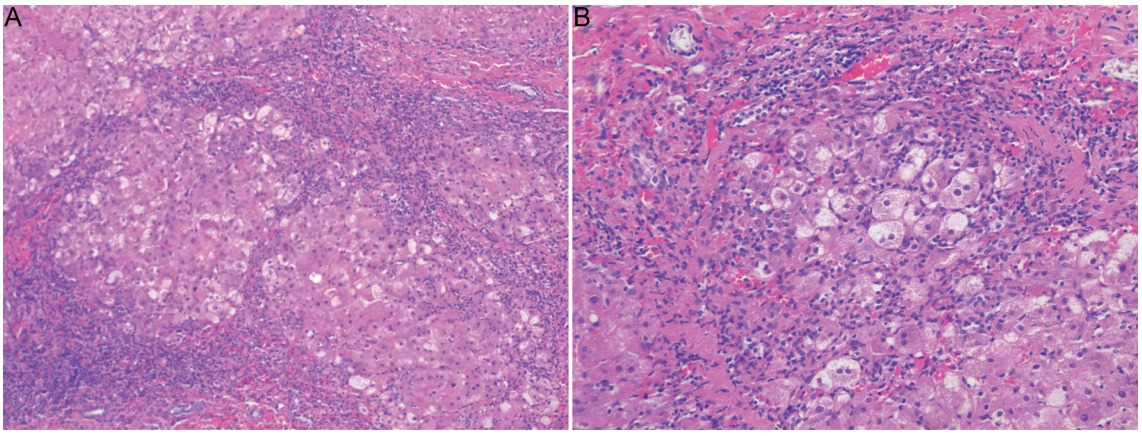

Fig. 2. Liver histological manifestations of WD patients. (A) and (B) show patient 1, a 42-year-old female. Liver tissue pathology (HE staining, 100×) showed "pseudolobules" with severe turbidity in the hepatocytes within the pseudolobules and macrovesicular fatty degeneration in some hepatocytes (F1-F2). There was mild to moderate lamina propria inflammation and "rosettes". The lesions showed active nodular cirrhosis (G2-3 S3-4). WD, wilson's disease; HE staining, hematoxylin-eosin staining.

47.37% vs. 3.45%) ($p < 0.05$). The cirrhosis group also had lower levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), white blood cells, and platelets ($p < 0.05$). The levels of gamma-glutamyl transferase, total bilirubin, hemoglobin, international normalized ratio, as well as the rate of males, serum ceruloplasmin score, urinary copper scores, c.2333G>T (p.Arg778Leu) mutation, and the number of mutation sites, showed no significant differences ($p > 0.05$) (Table 1). Among the 19 cirrhosis patients, 42.10% (8/19) were classified as Child-Pugh Class A, 31.58% (6/19) as Class B, and 26.32% (5/19) as Class C.

Risk factors for liver cirrhosis in Wilson's disease

Logistic regression analysis, considering age at diagnosis, ALT, AST, ALP, platelets, immunoglobulin G, and K-F ring at admission, was employed to determine risk factors for WD cirrhosis. Age at diagnosis (OR = 1.072, 95% CI = 1.007–1.142, $p = 0.03$) and K-F ring (OR = 18.657, 95% CI = 1.451–239.924, $p = 0.025$) were independently linked to WD cirrhosis (Table 2).

Risk factors associated with WD cirrhosis

Age at diagnosis had 89.5% sensitivity, 69.0% specificity, and an AUC of 0.802 (95% CI: 0.679–0.926) for diagnosing WD cirrhosis, with a cut-off value of 22 years old. In addition, the K-F ring showed 47.4% sensitivity, 96.6% specificity, and an AUC of 0.72 (95% CI: 0.56–0.879) for diagnosing WD cirrhosis. Combining the tests provided a diagnostic sensitivity of 100%, a specificity of 65.5%, and an AUC of 0.909 (95% CI: 0.831–0.988) for diagnosing WD cirrhosis (Fig. 3).

Treatment, follow-up, and prognosis of patients with WD hepatic type

Fifteen patients were lost to follow-up, and the average follow-up time for the remaining 33 patients was 48.6 months (12–72 months). Nineteen patients received zinc gluconate treatment and tolerated it well. Five patients (2%, 5/30) developed knee pain when taking five to seven tablets of penicillamine per day, but they were able to tolerate it by reducing the dose to three to six tablets per day. Another three patients (9.1%, 3/33) stopped taking penicillamine on the 3rd to 4th day after developing a large rash and were later switched to dimercaptosuccinic acid. Finally, 14 patients (42.42%) were treated with penicillamine alone, 16 patients (48.48%) were treated with zinc gluconate combined with penicillamine (three to eight tablets/day) for maintenance treatment, and three patients (9.09%) were treated with zinc gluconate combined with dimercaptosuccinic acid (two to four tablets/day) for maintenance treatment. The biochemical recovery rate for all patients was more than 60% after 12–72 months of treatment, and there was no significant difference between the different drug groups ($p > 0.05$). After 12 months of treatment, the 24-h urine copper content was significantly reduced compared to baseline, with no significant difference among the three groups ($p > 0.05$, Table 3). After 12 months of treatment, 84.62% of patients showed significant improvement in cirrhosis nodules on imaging (Fig. 4). The mean liver stiffness measurement of 13 patients with cirrhosis decreased from 15.3 ± 3.2 kPa to 8.7 ± 4.5 kPa.

During the follow-up period, one patient developed hepatocellular carcinoma six years after diagnosis, underwent surgical resection, and is still under follow-up. Three patients voluntarily stopped the drug for six months and later developed abnormal liver function or ascites again and resumed treatment. One patient developed breast cancer four years after diagnosis. There were no complications such as new ascites, neurological symptoms, hepatic encephalopathy, or upper gastrointestinal bleeding. No other obvious side effects requiring discontinuation of the drug were observed.

Discussion

Wilson's disease is a disorder of copper metabolism that is diagnosable, treatable, and even preventable.[24,25] However, due to the diversity of its clinical manifestations, the lack of distinct patterns in liver histology, and the complexity of genetic mutations (varying mutation sites, and even the same mutation presenting diverse clinical manifestations), the diagnosis of WD is frequently difficult.[25,26] Many WD patients do not receive a timely diagnosis and treatment, which can lead to progressive chronic hepatitis and cirrhosis. In this study, 48 patients with WD had liver damage, with three patients having neurologic involvement and one presenting with hemolysis. The patients ranged in age from two to 69 years, and the proportion of pediatric and adult patients was nearly equal, which better reflects the progression of the disease. Cirrhosis patients had an older age at diagnosis. At the same

Table 1. Comparison of clinical characteristics and laboratory data in the cirrhosis and non-cirrhosis groups

	All (n = 48)	Non-cirrhosis group (n = 29)	Cirrhosis group (n = 19)	*p*-value
Gender, male n (%)	26 (54.17%)	17 (58.62%)	9 (47.37%)	0.444
Age at diagnosis (y)	28 (10.25, 40.5)	12 (6, 34.5)	36 (28, 47)	<0.001
ALT (U/L)	79.05 (36.45, 168.65)	156.5 (76.1, 227.7)	37.5 (26.7, 63.9)	<0.001
AST (U/L)	79.7 (40.525, 107.45)	89.282 (61.4, 118.9)	39.5 (31.2, 83.1)	<0.001
ALP (U/L)	164.75 (94.25, 243.275)	193.6 (144, 286.85)	118.5 (82, 150)	0.003
GGT (U/L)	86.1 (43.75, 123.225)	103 (53, 121.75)	49 (30.9, 127)	0.067
TBIL (μmol/L)	16.15 (10.65, 44.275)	14.9 (8.9, 38.2)	19.4 (14.3, 48.125)	0.08
WBC (10^9/L)	5.0834 (3.61, 6.0275)	5.0834 (4.865, 6.2)	4.32 (3.27, 5.63)	0.048
PLT (10^9/L)	142 (78, 211)	156.512 (127.5, 271)	78 (53, 139)	0.002
Hb (g/L)	121.244 (112.5, 131.75)	125 (121.244, 131.5)	114 (103, 135)	0.074
IgG (g/L)	13.906 (10.425, 15.075)	13.906 (8.3, 13.906)	14.9 (13.6, 18.3)	0.004
INR	1.3189 (1.0725, 1.425)	1.24 (1.055, 1.3189)	1.43 (1.16, 1.63)	0.052
CER score				0.753
1 point, n (%)	19 (39.58%)	12 (41.38%)	7 (36.84%)	
2 points, n (%)	29 (60.42%)	17 (58.62%)	12 (63.16%)	
Urine Copper Score				0.394
1 point, n (%)	12 (25.00%)	9 (31.03%)	3 (15.79%)	
2 points, n (%)	36 (75.00%)	20 (68.97%)	16 (84.21%)	
K-F ring, n (%)	10 (20.83%)	1 (3.45%)	9 (47.37%)	0.001
p. Arg778Leu, n (%)	19 (41.30%)	12 (41.38%)	7 (41.18%)	0.989
Number of mutation sites, (n = 46)				0.183
1 site, n (%)	10 (21.74%)	2 (10.00%)	8 (30.77%)	
≥2 sites, n (%)	36 (78.26%)	18 (90.00%)	18 (69.23%)	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, glutamyltransferase; TBIL, total bilirubin; WBC, white blood cells; PLT, platelets; Hb, hemoglobin; IgG, immunoglobulins G; INR, international normalized ratio; CER score, Serum ceruloplasmin score; K-F ring, Kayser-Fleischer ring.

time, multivariate analysis confirmed that age at diagnosis and K-F ring were risk factors for WD cirrhosis. This could be because aberrant copper metabolism gradually accumulates from the liver into other tissues over time, primarily harming the cornea and nervous system.[27,28] This suggests that early detection and therapy are critical for preventing disease progression. Studies have shown that corneal K-F rings are an essential indicator in most neurologic patients, nearly half of hepatic patients, and around one-third of asymptomatic individuals.[29–31] This study identified that only 20.83% of patients exhibited K-F rings, but 90% of patients with cirrhosis showed them. The AUC for the combination of age at diagnosis and K-F rings was 0.909 (95% CI: 0.831–0.988), suggesting that this model possesses significant diagnostic value.

At the 8th International Conference on Wilson's Disease

Table 2. Univariate and multivariate analysis of the risk factors for the progression of Wilson's disease to cirrhosis

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Age at diagnosis (y)	1.07 (1.025, 1.117)	0.002	1.072 (1.007, 1.142)	0.03
ALT (U/L)	0.971(0.955, 0.988)	0.001		
AST (U/L)	0.966(0.945, 0.988)	0.003		
ALP (U/L)	0.986(0.976, 0.996)	0.005		
PLT (10^9/L)	0.989 (0.98, 0.997)	0.009		
IgG (g/L)	1.254 (1.05, 1.497)	0.012		
K-F ring	25.2 (2.824, 224.835)	0.004	18.657 (1.451, 239.924)	0.025

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; PLT, platelets; Hb, hemoglobin; IgG, immunoglobulins G; K-F ring, Kayser-Fleischer ring; OR, odds ratio; CI, confidence interval.

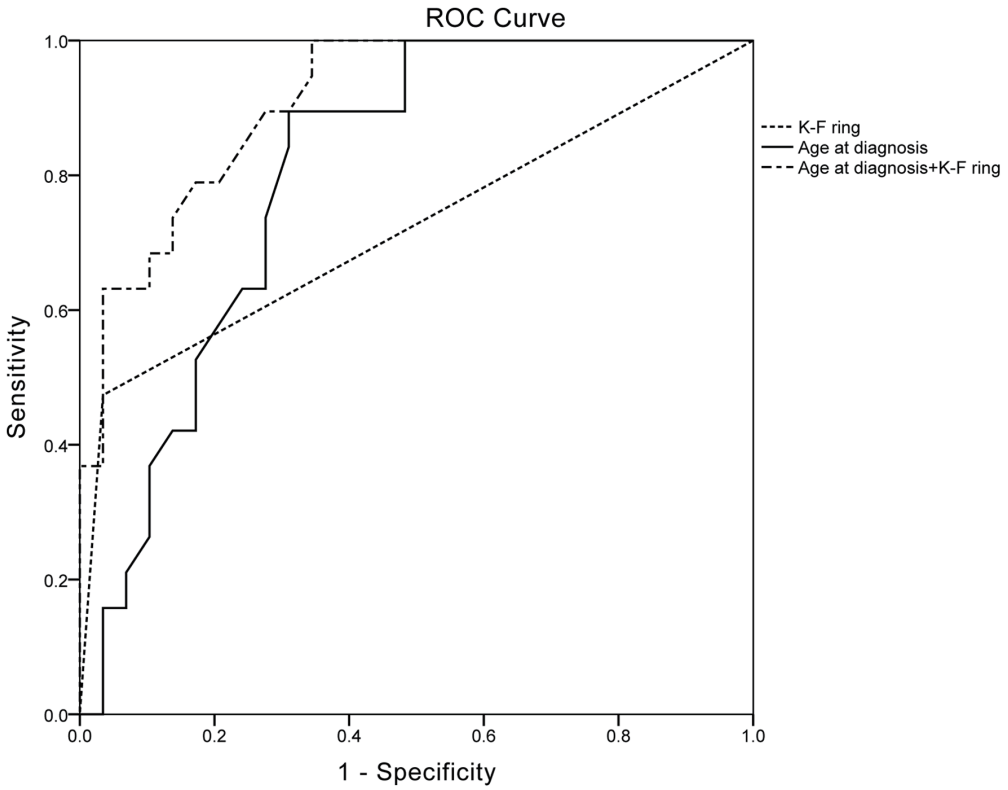

Fig. 3. ROC curve of relevant influencing factors. The prediction of WD cirrhosis was analyzed using an ROC curve with age at diagnosis, K-F ring, and the combination of age at diagnosis and K-F ring. The AUC values were 0.802, 0.72, and 0.909, respectively. ROC, receiver operating characteristic; AUC, area under the receiver operating characteristic curve; K-F ring, Kayser-Fleischer ring.

in Leipzig in 2001,[32] the working group's diagnostic scoring system for Wilson's Disease (the Leipzig scoring system) exhibited good diagnostic accuracy and has been extensively used. Due to the lack of a single test capable of confirming or excluding WD, diagnosis is frequently difficult and delayed. This study found that 54.17% of patients (26/48) could be diagnosed with Wilson's Disease (Leipzig score ≥ 4) based on ceruloplasmin, 24-h urinary copper, and K-F rings. Patients

Table 3. Improvement of patients' biochemical indicators and imaging after treatment

	All (n = 33)	Penicillamine (n = 14)	Penicillamine + zinc gluconate (n = 16)	Dimercaptosuccinic acid + zinc gluconate (n = 3)	p^*-value	*p*-value
Age at diagnosis (y)	28.00 (12.00, 36.00)	19.50 (8.75, 35.75)	28.00 (16.00, 36.00)	34.00 (20.00, 37.50)	0.270	0.556
Gender, male n (%)	15 (45.45)	6 (42.86)	8 (50.00)	1 (33.33)	0.730	0.886
Biochemical recovery rate n/n(%)						
12 months	29/33 (87.88)	12/14 (85.71)	15/16 (93.75)	2/3 (66.67)	0.586	0.276
24 months	22/25 (88.00)	8/10 (80.00)	11/12 (91.67)	3/3 (100.00)	0.571	0.713
36 months	19/22 (86.36)	6/8 (75.00)	11/12 (91.67)	2/2 (100.00)	0.537	0.657
48 months	15/17 (88.23)	6/6 (100.00)	9/11 (81.82)		0.515	
60 months	13/15 (86.67)	5/5 (100.00)	8/10 (80.00)		0.524	
72 months	6/8 (75)	3/3 (100.00)	3/5 (60.00)		0.464	
24-h urine copper level after 12 months of treatment(μg/24 h)	119.00 (80.77, 189.57)	147.50 (135.80, 268.00)	86.32 (69.80, 135.30)	-	0.147	
Radiographic improvement after 12 months of treatment n/n (%)	11/13 (84.62)	5/7 (85.71)	6/6 (100)		0.462	

p^*: comparison between penicillamine and penicillamine + zinc gluconate.

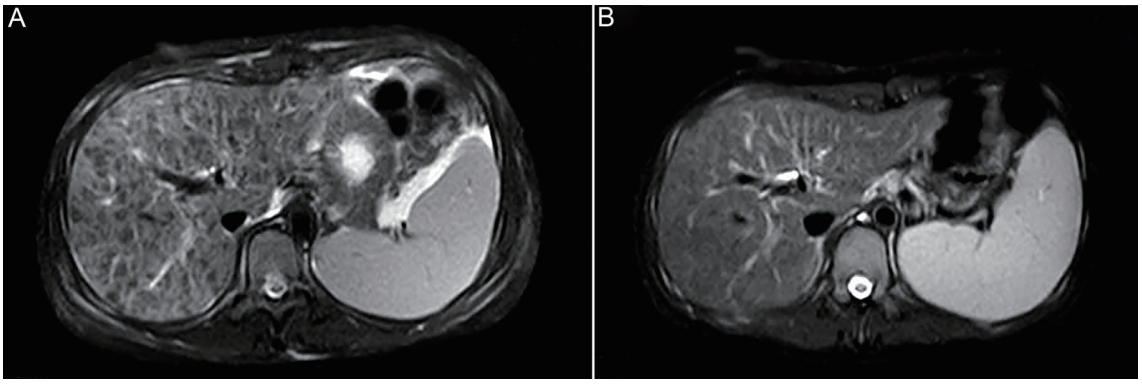

Fig. 4. Imaging findings of the patient before and after treatment. Magnetic resonance imaging of the upper abdomen of a 13-year-old girl. (A) T2 low-signal nodules with unclear boundaries, considered cirrhotic nodules; splenomegaly and ascites; (B) After one year of treatment with zinc gluconate (seven tablets/day) combined with penicillamine (four tablets/day), the T2 low-signal nodules were significantly reduced, and no ascites were seen.

with long-term abnormal liver function should prioritize testing for ceruloplasmin and 24-h urinary copper for exclusion. Ceruloplasmin levels showed no difference in the presence of cirrhosis, indicating a possible link with genetic alterations and no worsening with disease progression.

This study showed that 45.83% of patients required a diagnosis based on genetic results. A total of 42 mutations were detected in 46 patients who tested for the ATP7B gene. The most prevalent of which was c.2333G>T (p.Arg778Leu) (41.30%, 19/46), while two or more mutation sites were the most common (73.91%, 34/46). There was no difference in the c.2333G>T (p.Arg778Leu) mutation in terms of the presence of cirrhosis. This indicated that there is no significant correlation between genotype and clinical phenotype, which is consistent with other studies. Among the nine patients, only a single mutation site was identified; however, the Leipzig score for ceruloplasmin, 24-h urinary copper, and K-F rings reached 3 or above, allowing for a diagnosis through genetic combination. Genetic testing not only enhances the diagnostic rate for patients but also holds significant implications for eugenics.

This study reveals that the non-cirrhosis group had much higher levels of ALT and AST than those in the cirrhosis group, while the cirrhosis group had higher total bilirubin levels, indicating more serious liver damage. The reason could be long-term excessive copper deposition in the liver, which leads to oxidative stress and subsequent oxidative damage to cellular proteins, lipids, DNA, RNA, and mitochondria, resulting in liver cell damage and steatohepatitis.[33] Additionally, copper can activate hepatic stellate cells, hastening the process of liver fibrosis.[34,35] Studies also show that WD patients' pathological changes are characterized by steatosis, inflammation, and cell death.[35–38] Liver biopsies from WD patients exhibit ultrastructural changes, including significant mitochondrial alterations, an increased number of peroxisomes, and the presence of lipolysosomes—cytoplasmic bodies formed by lipid vacuoles encased in electron-dense lysosomes.[39,40] The liver tissue pathology of this study confirmed that 75% showed fatty degeneration, and steatosis grade ≥ 1 in 88.89% (8/9). 75% (9/12) had interface inflammation, and imaging also showed that 31.25% had fatty liver. Consequently, WD should consistently be considered in the differential diagnosis for children and young adults exhibiting abnormal liver function tests or signs of nonalcoholic fatty liver disease and autoimmune liver disease.

The key treatment for WD is to promote the excretion of copper and reduce its absorption. Currently, penicillamine, zinc agents, and dimercaptosuccinic acid are the most common copper-excretion drugs. Penicillamine is a thiol-containing amino acid that can effectively complex copper in the circulation. Zinc gluconate can prevent the absorption of copper. This study showed that zinc gluconate has few side effects and is well tolerated. The biochemical recovery rate of patients treated with zinc gluconate combined with penicillamine or dimercaptosuccinic acid for 12 to 70 months was over 60%, which is consistent with the effectiveness of penicillamine monotherapy (73.7%) reported by Bruha *et al*. and zinc gluconate monotherapy (71.6%) reported by Weiss *et al*.[41,42] After one year of copper removal treatment, the 24-h urinary copper content was significantly reduced compared with the baseline; 84.62% of patients' imaging showed significant improvement in cirrhotic nodules, and the mean liver stiffness measurement of 13 patients with cirrhosis decreased, indicating that the nodules caused by copper deposition and inflammatory damage gradually decreased. There was no phenomenon of brain-type patients stopping the drug due to worsening neurological symptoms in the early stage of penicillamine treatment. Overall, whether it is penicillamine alone or combined with zinc gluconate treatment, it is safe and effective for liver-type patients. In addition, during copper removal treatment, a 46-year-old male patient with cirrhosis developed hepatocellular carcinoma, and a 38-year-old woman developed breast cancer, indicating that copper deposition does not protect against the occurrence of cancer.[43] There are few reports of WD combined with liver cancer, colon cancer (one case), acute lymphoblastic leukemia (two cases), and breast cancer (one case).[43] The occurrence of cancer still needs to be monitored, especially liver cancer.

This is a retrospective, single-center study, but real-life cohort data encompass a broader range of patients compared to randomized controlled studies, which often exclude individuals with various comorbidities. As a result, these findings have greater relevance to ordinary clinical practice. It is required to increase the sample size to improve the research.

Conclusions

The hepatic symptoms of Wilson's disease are diverse, ranging from asymptomatic or accidental liver abnormalities to cirrhosis and acute liver failure, and they can onset at any age. The later the age of diagnosis, the greater the likelihood of developing cirrhosis. K-F rings are a reasonably independent clinical sign of WD; they may also represent the severity and progression of the disease. In addition, long-term cop-

per removal treatment is safe and effective. Early detection and early treatment play an important role in controlling the progression of the disease.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (QFX, YFY), acquisition of data (YJL, CSY), analysis and interpretation of data (YJL, CSY, YYM, KYO), drafting of the manuscript (YJL, CSY), critical revision of the manuscript for important intellectual content (QFX, YFY), administrative, technical, or material support (DXL, BL), and study supervision (QFX). All authors have made significant contributions to this study and have approved the final manuscript.

Ethical statement

The study protocol was approved by the Medical Ethics Committee of the Second Hospital of Nanjing (2024-LS-ky044; June 19, 2024) in accordance with the Helsinki Declaration as revised in 2013. The written consent form was obtained.

Data sharing statement

The datasets generated and analyzed during the present study are available from the corresponding author upon reasonable request.

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