

## CLINICAL SCIENCE

# Wilson's disease: an analysis of 28 Brazilian children

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**OBJECTIVES:** Clinical-laboratory and evolutionary analysis of twenty-eight patients with Wilson's disease.

**METHODS:** Twenty-eight children (twelve females and sixteen males) with Wilson's disease were evaluated retrospectively between 1987 and 2009, with a follow-up of 72 months (1 – 240 months). The clinical, laboratory, and histologic features at diagnosis were recorded at the end of the study.

**RESULTS:** The median age at diagnosis was 11 years (2 – 18 years). Twelve patients were asymptomatic, seven had hepatitis symptoms, five had raised aminotransferase levels, three had hepatomegaly associated with neurological disorders, one had fulminant hepatitis with hemolytic anemia, and six patients presented with a Kayser-Fleischer ring. A histological analysis revealed that six children had chronic hepatitis, seven had cirrhosis, two had steatosis, one had portal fibrosis, and one had massive necrosis. The treatment consisted of D-penicillamine associated with pyridoxine for 26 patients. Adverse effects were observed in the other two patients: one presented with uncontrollable vomiting and the other demonstrated elastosis perforans serpiginosa. At the end of the study, all 26 treated patients were asymptomatic. Twenty-four of the patients were treated with D-penicillamine and pyridoxine, and two were treated with trientine and zinc sulfate. A liver transplant was performed in one patient with fulminant hepatitis, but the final patient died 48 hours after admission to the intensive care unit.

**CONCLUSIONS:** Family screenings associated with early treatment are important in preventing Wilson's disease symptoms and potentially fatal disease progression. The study suggests that Wilson's disease must be ruled out in children older than two years presenting with abnormal levels of hepatic enzymes because of the heterogeneity of symptoms and the encouraging treatment results obtained so far.

**KEYWORDS:** Child; Therapy; Screening; Wilson's disease; Hepatic.

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## INTRODUCTION

Wilson's disease (WD) is a rare autosomal recessive disease that affects approximately 1 in 30,000 live births, with a predominance in males. The disease prevalence is higher in countries where consanguineous marriage is frequent, such as in Turkey and Greece (1).

WD is characterized by a disturbance in copper metabolism that leads to the accumulation of the metal in various tissues of the body (1), and it is caused by mutations in the *ATP7B* gene that encodes the ATP7B protein. This protein is essential for transporting and excreting excess copper; it is expressed mainly in the liver and kidney and, at lower levels, in the brain, lungs, and placenta. ATP7B is a transmembrane protein of the trans-Golgi network that incorporates free copper into the apoceruloplasmin molecule, which in turn

transports the excess metal to the excretory vesicles of the biliary ducts (2-6).

Copper accumulation triggers a variety of clinical manifestations that most often affect neurological and liver functions, although effects have also been observed for bone, retina, kidney, and hematological tissues. In general, hepatic manifestations tend to occur earlier in life, whereas neurological manifestations appear after adolescence (1,7). The symptoms rarely appear before the age of three years, and the overall clinical picture varies from asymptomatic to acute liver failure. Additionally, hemolysis may occur with the formation of gallstones from the bilirubinate calculations (2).

This disease is diagnosed through clinical and family history analysis. Methods associated with the diagnosis include physical examinations such as ophthalmic examinations, biochemical tests including the measurement of 24-hour urinary copper levels after a penicillamine challenge test, and analyses for genetic mutations (3,8-10). WD treatment is based on copper chelation and symptom control; however, in more advanced cases when liver failure occurs, liver transplantation is the only effective treatment (2,11).

Few reports have evaluated the clinical course of adult Brazilian patients with WD (12), and only one study

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preliminarily evaluated pediatric patients (13). The aim of this study was to analyze the clinical and laboratory evolutions of WD and the effects of the standard treatments in Brazilian children with WD.

**PATIENTS AND METHODS**

A retrospective study of 28 children (12 females and 16 males) with WD was conducted between 1987 and 2009. These patients were referred to the Hepatology Unit at the Children's Institute at the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo. The age at diagnosis ranged from 2 to 18 years (median age 11 years).

The study was approved by the Ethics Commission for the Analysis of Research Projects of our institution under protocol number 1026/09. Written informed consent was obtained from the parents or guardians.

The diagnoses of WD were based on clinical and biochemical parameters. We considered patients presenting at least two of the following parameters to be positive for the disease: levels of ceruloplasmin lower than 20 mg/ml in two separate measurements, a 24-hour urinary copper level higher than 100 µg/24 h, or the presence of a Kayser-Fleischer ring. Asymptomatic patients with low levels of ceruloplasmin but with first-degree relatives (siblings or parents) with WD were also included in the study (2).

Data obtained from medical records included medical and family history as well as clinical examination, including neurological assessments, presence of jaundice, hepatosplenomegaly, signs of liver failure, and the presence of a Kayser-Fleischer ring through a slit lamp. Biochemical laboratory tests evaluated at the beginning and end of the study were: hemoglobin (Hb) (g/dl), hematocrit (Ht) (%), white cell count (WCC) (/mm<sup>3</sup>), platelets (plat) (/mm<sup>3</sup>), aspartate aminotransferase (AST) (U/L), alanine transaminase (ALT) (U/L), gamma-glutamyltransferase (GGT) (U/L), alkaline phosphatase (APhos) (U/L), international normalized ratio (INR), total bilirubin (TB), direct bilirubin (DB), albumin (Alb) (g/dl), ceruloplasmin (mg/ml), 24-hour urinary copper levels (µg), urinary copper levels after a penicillamine challenge test (14 patients), abdominal ultrasonography, and liver biopsy. The ceruloplasmin levels in the serum were measured by radial immunodiffusion (normal range 20–60 mg/dL). The urinary copper and urinary copper after D-penicillamine sensitization were measured after the urine was collected in an acid-washed, plastic, metal-free container. The sensitization test with D-penicillamine was performed after the patients ingested 500 mg of D-penicillamine at time zero and again after 12 hours while the 24-hour urinary copper collection progressed. The copper levels in the urine were determined by atomic absorption spectrophotometry.

Histopathological analyses were performed on seventeen patients using reticulin staining, Masson trichrome stains, hematoxylin and eosin, and the qualitative detection of copper in the tissues. All patients selected for the study were excluded for other diseases such as viral hepatitis, autoimmune hepatitis, deficiency of alpha-1 antitrypsin, and illicit drug use.

**Treatment**

The initial treatment consisted of D-penicillamine (20 mg/kg/day) and pyridoxine (25–50 mg/day) for the

**Table 1 - Clinical and biochemical data obtained from the Wilsons disease patients (n = 28).**

Family screening	12 (42.8%)
Abnormal hepatic enzymes	5 (17.8%)
Symptoms of hepatitis	7 (25%)
Hepatosplenomegaly + neurological symptoms	3 (10.7%)
Fulminant hepatitis + hemolytic anemia (Coombs positive)	1 (3.5%)

26 patients. Trientine (550-750 mg/day) and zinc sulfate (300–450 mg/day) were administered to patients who presented adverse reactions to D-penicillamine.

**Data analysis**

The laboratory data were analyzed using SAS (Statistical Analysis System, version 9.3, SAS Institute, Cary, NC, USA). A t-test was used to evaluate the mean change in the laboratory tests from the baseline to the end of the study.

**RESULTS**

The analyses of the medical records of 28 WD patients are presented in Table 1. The data revealed that 12 (42.8%) of the patients were asymptomatic (diagnosed by family screening), seven (25.0%) presented clinical features of hepatitis, one (3.5%) had signs of liver failure and acute hemolytic anemia with a positive Coombs test, five (17.8%) had abnormal liver enzymes and biochemical test results, and three (10.7%) had hepatosplenomegaly and neurological signs, such as cerebellar disturbances, dysarthria, tremors, and postural instability. In six patients (21.8%), we observed the presence of a Kayser-Fleischer ring and of these patients, four (66.7%) had signs of hepatitis, one (16.7%) displayed liver failure and hemolytic anemia, and one (16.7%) was asymptomatic. Table 1 shows the clinical and biochemical data from the patients when they entered in study. Table 2 shows the clinical manifestations of the 16 symptomatic patients diagnosed with WD. Table 3 shows the diagnostic biochemical laboratory results collected initially and those collected at the end of the study. The tests that showed significant results were: Hb ( $p = 0.0014$ ), Ht ( $p = 0.0062$ ), AST ( $p < 0.0001$ ), ALT ( $p < 0.0002$ ), GGT ( $p < 0.0026$ ), DB ( $p = 0.0041$ ), APhos ( $p = 0.0005$ ), and Alb ( $p = 0.0374$ ) (Table 4).

The median and range of the ceruloplasmin levels were 3 mg/dl (1–11 mg/dl). In 22 (78.5%) patients who underwent the 24-hour urinary copper test, the levels were 312 (53–2294) µg/24 h. In 6 cases, the diagnosis was based on the presence of low levels of ceruloplasmin; three of these patients (50.0%) displayed a Kayser-Fleischer ring, and the

**Table 2 - Clinical manifestations of symptomatic Wilsons disease patients at diagnosis (n = 16).**

Jaundice	8 (50%)
Hepatosplenomegaly	7 (43.7%)
Abdominal pain	6 (37.5%)
Vomiting	5 (31.2%)
Fatigue	3 (18.7%)
Choluria	7 (43.7%)
Hipochohla/Achohla	4 (25%)
Ascites	6 (37.5%)
Edema of lower limbs	6 (37.5%)
GI bleeding	3 (18.7%)

Abbreviation: Gastrointestinal bleeding (GI bleeding).

**Table 3 - Laboratory findings of the WD patients at time of diagnosis and at the end of the study.**

Variable	No. of Patients	N	mean	std	median	Min	max
Hb(g/dl)	basal	27	12.45	1.54	12.60	6.10	14.60
	end of study	27	13.62	2.38	13.50	6.80	19.70
Ht(%)	basal	27	37.24	4.81	38.30	17.10	43.50
	end of study	27	39.56	6.18	40.00	17.30	49.40
WBC(/mm <sup>3</sup> )	basal	25	6254.00	2801.04	6600.00	2200.00	13500.00
	end of study	26	5930.00	2528.08	5800.00	2600.00	15100.00
Plat(/mm <sup>3</sup> )	basal	26	236000.00	131970.75	210000.00	70000.00	531000.00
	end of study	26	211000.00	119184.64	184000.00	32000.00	428000.00
AST(U/L)	basal	26	89.42	65.21	69.50	15.00	301.00
	end of study	26	25.46	13.89	21.50	10.00	73.00
ALT(U/L)	basal	26	127.00	112.30	100.00	16.00	457.00
	end of study	26	37.00	25.58	33.50	7.00	105.00
GGT(U/L)	basal	26	85.98	105.20	50.75	15.00	530.00
	end of study	25	30.38	29.88	24.00	7.00	160.00
APhos(U/L)	basal	25	464.80	264.43	380.00	67.00	1054.00
	end of study	25	256.50	209.57	169.00	41.00	885.00
TB(g/dl)	basal	23	1.04	0.70	0.70	0.31	3.00
	end of study	23	0.85	0.51	0.79	0.11	2.20
DB(g/dl)	basal	23	0.45	0.40	0.30	0.05	1.70
	end of study	23	0.19	0.10	0.20	0.02	0.40
Alb(g/dl)	basal	19	3.79	0.93	3.80	1.50	5.30
	end of study	19	4.31	0.47	4.27	3.10	5.53
INR	basal	21	1.34	0.26	1.25	0.97	1.91
	end of study	21	1.24	0.21	1.14	1.01	1.68

Abbreviations: hemoglobin (HB) (g/dl), hematocrit (HT) (%), white cell count (WCC) (/mm<sup>3</sup>), Platelets (PLAT) (/mm<sup>3</sup>), aspartate aminotransferase (AST) (U/L), alanine transaminase (ALT) (U/L), gamma-glutamyltransferase (GGT) (U/L), alkaline phosphatase (APhos) (U/L), total bilirubin (TB) (mg/dl), albumin (Alb) (g/dl), international normalized ratio (INR).

other three (50.0%) were siblings of WD patients. In 11 patients displaying normal urinary copper levels, the test for sensitization with D-penicillamine revealed urinary copper levels that were higher than 1,600 µg/24 h.

Histopathology revealed portal fibrosis in one patient (5.8%), massive necrosis in one patient (5.8%), liver steatosis in two patients (11.7%), chronic hepatitis in six patients (35.3%), and cirrhosis in seven patients (41.1%).

Twenty-six patients began treatment with D-penicillamine and pyridoxine. However, two of the patients (7.7%) had adverse effects: one had uncontrollable vomiting after four months of treatment, and the other had elastosis perforans serpiginosa (EPS) after 72 months. The patients were then treated with trientine and zinc sulfate, which produced a good outcome in both patients. In five patients (19.2%) with no adverse effects, zinc sulfate was added to the D-penicillamine treatment because although D-penicillamine

preserved hepatic function, the liver enzymes continued to be abnormal after one year of treatment. The introduction of zinc sulfate normalized the liver enzymes of four patients, and one patient remained with minimal change. At the end of the study, 24 patients showed normalized levels of liver enzymes and only two continued to present abnormal results. These two patients had preserved liver functions. Nineteen patients continued the medication initially prescribed for an average interval of 56.4 months (ranging from 4 to 120 months), and all of them achieved eradication of the WD symptoms and demonstrated normal laboratory test results.

The mean follow-up at the end of the study was 72 months (one patient was followed for 240 months). Twenty-six patients (92.8%) remained asymptomatic. Of these patients, 24 (92.3%) displayed normal liver enzymes, but two (7.7%) showed abnormal liver enzymes while maintaining normal hepatic function. We observed a significant normalization of the hematological and biochemical test results.

We detected complications in some patients during the course of the disease and treatment. One patient had bilateral renal lithiasis associated with hydronephrosis two years after the diagnosis, and one patient had micro and macroscopic hematuria. One patient underwent liver transplantation and was followed up for 18 months (end of the study), and one patient died 48 hours after entry into the ICU for sepsis. This patient had severe hepatic failure, hepatic encephalopathy, and sepsis. The diagnosis of WD was based on lab test results that arrived after the time of death.

**DISCUSSION**

Studies of WD in childhood are limited because the number of patients available to compose a relevant study is

**Table 4 - Laboratory data – Change from baseline.**

Variable	No. of Patients	Pr>F (t test)*
Hb(g/dl)	27	0.0014
Ht(g/dl)	27	0.0062
AST(U/L)	26	<0.0001
ALT(U/L)	26	<0.0002
GGT(U/L)	26	<0.0026
TB(g/dl)	23	0.1998
DB(g/dl)	23	0.0041
WBC(/mm <sup>3</sup> )	25	0.5396
INR	21	0.2578
Aphos(U/L)	25	0.0005
Plat(/mm <sup>3</sup> )	26	0.1549
Alb(g/dl)	19	0.0374

\*t test for the hypothesis: H<sub>0</sub>: mean change (end of the study – baseline) = 0.

frequently low, which precludes large cohort studies or randomized controlled trials. Although reports on pediatric WD patients from other countries have been published, only one study has been published on Brazilian children (13). Machado et al. (2006) evaluated 119 Brazilian patients, mostly adults, with a mean age at diagnosis of 19.3 [7–37] and reported that the major initial clinical manifestation was neurological (12). In our study, patients were diagnosed at a mean age of 10 years, and most did not have any neurological symptoms.

The diagnosis of WD is usually established by biochemical testing following clinical suspicion. There is no single diagnostic test that can exclude or confirm WD with certainty. A diagnosis of WD should be based on a combination of clinical features, laboratory findings, and mutation analysis (1). For cases of doubtful diagnosis even after biochemical testing, molecular genetic analyses are used to identify disease-causing alleles (14). In our study, most of the cases were clinically diagnosed after three years of age, and only those with a family history were screened and had a diagnosis confirmed earlier. Although we did not perform the genetic test, the data from the literature do not show a correlation between genotype and age at diagnosis, serum ceruloplasmin levels, presence of cirrhosis or disease (1).

One of the clinical criteria for the diagnosis of WD is the Kayser-Fleischer ring, which is more common in adults than in children (15). Although this is considered a strong sign, only 21.4% of the patients in our study actually presented with the ring, and only one showed any neurological symptoms associated with the ring. This result is in accordance with results of other studies (16-18).

The biochemically abnormal ceruloplasmin levels helped us to diagnose the disease, but normal values are frequently observed in WD patients (1,10). Indeed, we found that four patients (14.2%) showed normal ceruloplasmin values, and the diagnosis was only possible because we detected high levels of urinary copper after the 24-hour urinary copper test, observed the presence of the Kayser Fleischer ring, or observed improvement in the liver enzymes after the initiation of treatment. Sometimes, even with reduced levels of ceruloplasmin, the 24-hour urinary copper levels are not always elevated. When this happens, sensitization with D-penicillamine is one way to confirm the diagnosis (10,17-19). A study by Muller et al. comprising 38 children with WD found that sensitization with D-penicillamine had a specificity of 92.8% and a sensitivity of 88.2% (19).

WD encompasses asymptomatic patients, patients presenting changes in biochemical tests only, and patients with acute liver failure, both with and without hemolytic anemia. The clinical features in our study were mild and different from those of other pediatric reports, which have mostly included WD children with acute liver failure or chronic symptomatic liver disease (2,10,14,18). This heterogeneity of WD symptoms, and the encouraging treatment results obtained so far, suggest that families and clinicians should be watchful of patients older than three years. In our study, all symptomatic patients presented initial signs of liver involvement or had abnormal liver enzyme measurements, which was expected given the history of the disease and the age of the patients. We show in this study that patient screening based on a family history of WD can enable early diagnosis before the onset of symptoms, thereby leading to a successful outcome following treatment.

We observed a low percentage of patients with neurological symptoms, which was expected because such characteristics are most commonly found in adults (20). For pediatric WD patients, deterioration in school performance, impulsive behavior, and even a loss of motor coordination, dysarthria, dystonia, spasticity, tremor, depression, psychosis, and personality disorders have been reported (11,12,15).

A retrospective study involving 25 pediatric WD patients with renal symptoms revealed that such manifestations are often undiagnosed and poorly reported and that they mostly present later in the course of the disease (21). These findings may be explained by the disease itself as well as the adverse effects of D-penicillamine (21,22). In all cases, it is advisable to perform total abdominal ultrasound and renal function biochemical tests to help diagnose and follow up the patients. In our study, renal manifestations were unusual.

The drugs available to treat WD are D-penicillamine, trientine, and zinc. The treatment is life-long and is usually associated with a diet low in copper. The most potent drug, D-penicillamine, is a derivative of penicillin that forms a soluble copper compound that can then be eliminated in the urine (23). Deficiency of copper arising from the treatment was not observed in any of our patients, in agreement with the findings in the literature (24). Despite its chelating action, D-penicillamine may induce metallothionein in the liver, leading to reduced copper levels and consequently reduced toxic effects (24). Because D-penicillamine can cause a deficiency in pyridoxine levels, a prophylactic dose of vitamin B6 is usually given to WD patients being treated with this drug (25).

Vega et al. analyzed children with WD after one year of treatment with D-penicillamine and found a normalization of the enzymatic test. However, they also showed that in patients with severe disease, normalization of albumin levels and coagulation are obtained later in the course of the treatment (24).

In our study, two patients were treated with trientine as an alternative therapy to D-penicillamine. Although it is a less potent chelator, trientine is recommended in case of adverse reactions to D-penicillamine (24). Another alternative drug is zinc, which interferes with the intestinal absorption of copper. In enterocytes and hepatocytes, zinc induces the synthesis of a copper-binding ligand, probably a thionein, which sequesters copper from the nutrient medium, making it unavailable for serosal transfer (24). This complex is then eliminated in the shedding of the gastrointestinal tissue. Currently, zinc is used to maintain asymptomatic patients with prolonged use of D-penicillamine or trientine (18). Brewer et al. (2005) reported a successful experience using zinc acetate to treat a group of 34 children (26). The results presented here also show a satisfactory response to WD treatment using zinc.

Regarding the adverse reactions induced by D-penicillamine, Vega et al. reported that toxic effects of the drug affected 10–20% of patients, usually early on in the treatment (24). One of the common adverse reactions observed is EPS, which usually presents as annular lesions consisting of hyperkeratotic reddish-brown papules arranged in a semi-circle, with a central area of atrophic skin (27,28). EPS affects mainly the patient's neck and upper limbs, and less frequently the axils, thighs, heels, and penis (28). In most cases, EPS occurs after long periods of use (mean 11.3 years) of D-penicillamine (29-31). EPS may be stopped by the

administration of zinc and trientine, as suggested by Bécuwe et al. (27). The presence of EPS is unusual in pediatric patients. We did, however, observe a single patient with this adverse effect following the use of D-penicillamine for 5 years. We proceeded with monotherapy with zinc sulfate, which produced good outcomes as the patient showed no worsening of clinical signs or laboratory findings. However, although treatments with zinc salts can be effective in most patients with WD, chelating agents are better at preventing hepatic deterioration (27).

Liver transplantation (LT) can save the lives of patients with WD who present with hepatic failure or chronic liver disease and are unresponsive to other medical treatments. Few reports have evaluated the outcomes of children and adults after LT for WD. Recently, Arnon et al. demonstrated that LT was an excellent treatment option for patients with WD (32). In our study, the single patient that received liver transplantation showed a favorable outcome.

We conclude that WD should be diagnosed by considering the family and clinical history as well as information from the biochemical laboratory tests because of the heterogeneity of the symptoms and the occurrence of asymptomatic patients. Additionally, children who are older than two years and have a family history of WD or increased hepatic enzyme levels and symptoms of acute hepatitis without any etiological viral markers or autoimmunity should be routinely screened for WD. Early diagnosis accompanied by the initiation of therapy with copper chelators, zinc salts, or even liver transplantation are essential for favorable outcomes, such as preventing morbidity and mortality.

## AUTHOR CONTRIBUTIONS

Kleine RT and Mendes RF organized all records in the tables, performed the analysis and wrote the manuscript. Miura IK, Pugliese RP, Danesi VL and Porta G assisted all the patients during the study and helped in the literature revision and manuscript writing.

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