CLINICAL SCIENCE

Wilson's disease in southern Brazil: a 40-year follow-up study

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BACKGROUND: Long-term data on the clinical follow-up and the treatment effectiveness of Wilson's disease are limited because of the low disease frequency. This study evaluated a retrospective cohort of Wilson's disease patients from southern Brazil during a 40-year follow-up period.

METHODS: Thirty-six Wilson's disease patients, diagnosed from 1971 to 2010, were retrospectively evaluated according to their clinical presentation, epidemiological and social features, response to therapy and outcome.

RESULTS: Examining the patients' continental origins showed that 74.5% had a European ancestor. The mean age at the initial symptom presentation was 23.3 $\pm$ 9.3 years, with a delay of 27.5 $\pm$ 41.9 months until definitive diagnosis. At presentation, hepatic symptoms were predominant (38.9%), followed by mixed symptoms (hepatic and neuropsychiatric) (30.6%) and neuropsychiatric symptoms (25%). Kayser-Fleischer rings were identified in 55.6% of patients, with a higher frequency among those patients with neuropsychiatric symptoms (77.8%). Eighteen patients developed neuropsychiatric features, most commonly cerebellar syndrome. Neuroradiological imaging abnormalities were observed in 72.2% of these patients. Chronic liver disease was detected in 68% of the patients with hepatic symptoms. 94.2% of all the patients were treated with D-penicillamine for a mean time of 129.9 $\pm$ 108.3 months. Other treatments included zinc salts, combined therapy and liver transplantation. After initiating therapy, 78.8% of the patients had a stable or improved outcome, and the overall survival rate was 90.1%.

CONCLUSION: This study is the first retrospective description of a population of Wilson's disease patients of mainly European continental origin who live in southern Brazil. Wilson's disease is treatable if correctly diagnosed, and an adequate quality of life can be achieved, resulting in a long overall survival.

KEYWORDS: Genetic and inherited disorders; Wilson's disease; treatment; medication; outcome.

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INTRODUCTION

Wilson's disease (WD) is an autosomal recessive inherited disorder of copper metabolism that leads to the accumulation of this metal in different organs and tissues. Hepatic and neurological symptoms are the main clinical features of the disease.

In 1912, Wilson first described this disorder as a familial disorder associated with neurologic symptoms and cirrhosis.[1] In 1956, Walshe first treated patients with the chelating agent D-penicillamine.[2]

The worldwide prevalence rate ranges from 10 to 30 cases per million, with increased rates in areas of consanguinity among parents.[3] The heterozygote carrier rate is 1 case per 100 persons, corresponding to a gene frequency varying between 0.3% and 0.7%.[4]

In WD, the processes of incorporating copper into ceruloplasmin and excreting excess copper into bile are impaired. The transport of copper by the copper-transporting P-type ATPase is defective secondary to one of several mutations in the *ATP7B* gene, which is localized in chromosome arm 13q.[5-7] This illness involves the loss of the ability to export copper from the liver into the bile and to incorporate copper into hepatic ceruloplasmin. As a consequence, copper accumulates in the liver, brain, kidneys and corneas.

The gene for WD is a cation-transporting P-type ATPase with 14 domains: 6 copper-binding domains, 4 transmembrane domains, 1 phosphatase domain, 1 transduction

domain, 1 phosphorylation domain, and 1 adenosine triphosphate (ATP)-binding domain. About 24.6% of all mutations involve the ATP-binding domain.[5]

Patients with WD usually present with liver disease between the first and second decades of life, whereas neurological and psychiatric symptoms develop later.[10] The diagnosis is confirmed by the measurements of serum ceruloplasmin, urinary copper excretion, and hepatic copper content, as well as the detection of Kayser-Fleischer rings.[9] It is important to diagnose Wilson's disease early, before severe damage occurs. Some researchers estimate that at least half of Wilson's disease patients are never diagnosed and die because of the untreated disease.[10]

The aim of this retrospective report is to analyze the clinical presentation, epidemiological and social features, the disease course and the response to therapy in a cohort of WD patients.

PATIENTS AND METHODS

Thirty-six patients with WD who were diagnosed from 1971 to 2010 and followed at the Departments of Neurology and Hepatology of a tertiary academic center in southern Brazil were reviewed. Clinical, biochemical, histological, radiological, epidemiological and social data were extracted from patient files and telephone contact.

The diagnosis of WD was based on clinical presentation, familial history, Kayser-Fleischer rings detected by slit lamp, low serum ceruloplasmin, abnormally high 24-h urinary copper excretion, liver biopsy, a genetic test and/or response to a D-penicillamine provocative test. Because this cohort was retrospective, a diagnostic pattern could not be determined for the whole group, although all 36 patients were highly likely to be diagnosed with WD.

Patients were classified according to their age, sex, family history and record of consanguinity among parents. Whenever available, the patients' continental origin was obtained based on their knowledge of their furthest ancestral countries of origin. This information was later classified as being purely European or a mixed continental origin of Europe and America. Patients with the mixed continental origin were investigated to determine the presence of a native indigenous ancestor.

Symptoms of initial presentation were classified into four major groups: exclusively hepatic symptoms, exclusively neuropsychiatric symptoms, hepatic and neuropsychiatric symptoms (mixed presentation) and asymptomatic individuals. In addition, patients were evaluated according to the clinical, radiological and histological investigations during the follow-up period.

The age at initial symptoms presentation was determined. The time between the initial symptoms and the definitive diagnosis was calculated in months for each patient.

Patients with liver disease were classified with chronic hepatopathy with or without portal hypertension, the presence of cirrhosis and the Child-Pugh classification for cirrhosis.[11] A liver biopsy was performed, when indicated, to elucidate diagnosis. Patients with neuropsychiatric disease were classified according to their clinical examination and radiological data.

Among patients who used D-penicillamine, the period of treatment was calculated in months. Response to therapy was determined based on clinical, laboratory, histological and radiological data.

Epidemiological and social features were determined based on the patients' degree of instruction, marital status, work skills and reproductive history.

A written informed consent form, approved by the local ethic committee, was used for this study. All data are presented as means and standard deviations. Statistical analysis was performed using Student's *t-test* and Fisher's exact test. A p value less than 0.05 was defined as statistically significant.

RESULTS

Baseline Characteristics

This cohort consisted of 36 WD patients, 16 male (44.4%) and 20 female (56.6%), with a mean age of 34.6 $\pm$ 10.8 (12–63) years. Family history was obtained for 16 (44.4%) patients, and only two individuals described a history of consanguinity among parents (5.5%).

Data about the patients' continental origins was available for 31 patients (86.2%). All the original countries from their oldest known ancestors were analyzed and included 16 (44.4%) patients from Italy, 8 (22.2%) patients from Poland, 8 (22.2%) patients from Brazil, 7 (19.4%) patients from Portugal, 5 (13.8%) patients from Germany, 3 (8.3%) patients from Spain, 3 (8.3%) patients from Switzerland, 2 (5.6%) patients from Ukraine, 1 (2.7%) patient from Russia and 1 (2.7%) patient from Ireland. This cohort presented a particularly high predominance of an exclusively European continental origin (n = 23, 74.5%). Eight (25.8%) subjects reported a mixed continental origin, with ancestors from Europe and America. Among these mixed continental origin patients, 3 (37.5%) subjects identified the presence of a native indigenous ancestor.

The mean age at the initial symptom presentation was 23.2 $\pm$ 9.3 (10–41) years. The time between the first clinical symptom and the definitive diagnosis was 27.5 $\pm$ 41.9 (1–194) months.

Clinical Features

At presentation, 9 patients (25%) showed exclusively neuropsychiatric symptoms, 14 (38.9%) patients exclusively hepatic symptoms, 11 (30.6%) patients demonstrated both hepatic and neuropsychiatric features (mixed presentation) and 2 (5.5%) patients were asymptomatic. The diagnosis among asymptomatic subjects was achieved through genetic family screening.

The age at the initial symptom was not different among the group of patients with exclusively neuropsychiatric symptoms (21.1 $\pm$ 8.9 years), exclusively hepatic symptoms (20.6 $\pm$ 7.2 years) or a mixed presentation (26.8 $\pm$ 9 years).

The mean time between the first clinical symptom and the definitive diagnosis was 18.5 $\pm$ 12.4 (1–36) months for patients with exclusively neuropsychiatric symptoms, 6.7 $\pm$ 5.8 (1–16) months for patients with exclusively hepatic symptoms and 51 $\pm$ 59.6 (1–194) months for patients with a mixed presentation. A statistical analysis showed a significant difference in the time required for the definitive diagnosis between patients with exclusively neuropsychiatric symptoms and exclusively hepatic symptoms (p = 0.02). There was no reduction in the time lag from the appearance of the first symptoms to the diagnosis in the more recent decades of this 40-year period of analysis.

The major findings of the initial clinical-laboratorial evaluation are presented in table 1. The patients were further

Table 1 - Clinical-Laboratory Findings (n = 36).

Neurological/Psychiatric	Hepatological	Ophtalmological	Renal	Hematological	Other Findings
Tremor of extremities (27.7%)	Cirrhosis (44.4%)	Kayser-Fleischer ring (55.6%)	Hypercalciuria (11.1%)	Hemolytic anemia (16.6%)	Arthralgia (5.5%)
Dysarthria (25%)	Portal hypertension (30.5%)		Kidney stones (8.3%)	Thrombocytopenia (5.5%)	Morning stiffness (5.5%)
Cerebellar disturbances (13.9%)	Jaundice (16.6%)				Skin hyperpigmentation (11.1%)
Dystonia (11.1%)	Astheny (8.3%)				Hair rarefaction (8.3%)
Parkinsonism (11.1%)	Acute liver failure (11.1%)				Asymptomatic (5.5%)
Others (headache/syncope) (5.5%)	Elevated liver transaminases (2.7%)				
Psychiatric (bipolar disorder, attention deficit disorder, personality alterations, body negligence, irritability, hypersomnia) (16.6%)	High digestive hemorrhage (5.5%)				

assessed according to their physical exam, neuroradiological imaging and histological findings.

Kayser-Fleischer Ring Analysis

Kayser-Fleischer rings were identified in 20 (55.6%) of the 36 subjects. These rings were most prevalent in patients with exclusively neuropsychiatric symptoms at presentation (77.8%), followed by patients with the mixed disease presentation (72.2%). Rings were least prevalent in subjects with exclusively hepatic symptoms (35.7%). Statistical analysis showed no significant differences when comparing the exclusively hepatic disease group and the neuropsychiatric group ($p = 0.08$) or the mixed disease group ($p = 0.11$). Both asymptomatic patients did not present Kayser-Fleischer rings.

Neuroradiological Imaging

Eighteen patients presented or developed neuropsychiatric features during the follow-up observation. All of them were submitted to a neuroradiological analysis with tomography or magnetic resonance imaging (MRI). Thirteen (72.2%) patients showed abnormalities previously described among WD neuropsychiatric patients. Major findings included brain atrophy with basal ganglia hyperintensities. In one patient, the MRI revealed the ''face of the giant panda'' sign that is characteristic of WD.

Liver Disease Evaluation

All 36 patients were submitted to an abdominal ultrasound. Chronic liver disease features were detected in 17 (68%) of the 25 patients with hepatic symptoms and only in 3 (27.2%) of the remaining subjects ($p = 0.03$). Among the 20 patients with ultrasound abnormalities, portal hypertension was demonstrated in 13 (65%) cases.

Sixteen patients with hepatic symptoms were submitted to a liver biopsy, and the histological findings are described in table 2.

Cirrhotic patients (n = 15) were classified according to the Child-Pugh Classification: 73.4% of patients were Child-Pugh A, and 26.6% of patients were Child-Pugh B or C (equal proportions of each group).

Treatment

Thirty-five patients were submitted to pharmacological treatment. Initially, 33 (94.2%) subjects were treated with D-penicillamine, and only two (5.8%) subjects were treated with zinc acetate.

One of the patients started on zinc acetate was an asymptomatic teenager diagnosed through genetic family screening, and the other one was a young woman with a fast progressive neurological disease for whom D-penicillamine treatment could be potentially deleterious as the first choice of therapy.

Among the group of patients who were started on D-penicillamine, therapy had to be switched to zinc acetate in 3 (9.1%) subjects. The reasons included pregnancy (1 subject) and the development of severe Parkinsonism, which improved after D-penicillamine treatment discontinuation (1 subject). The third patient had the D-penicillamine treatment stopped altogether for his own safety because he presented a significant nonadherence to the medication posology and poor treatment compliance.

Table 2 - Hepatic Histological Findings (n = 16).

Subject	Gender	Age	Histological Findings
1	M	26	Micronodular cirrhosis with mononuclear cellular infiltrate
2	F	17	Postnecrotic cirrhosis
3	F	29	Mild mononuclear cellular infiltrate
4	F	21	Postnecrotic cirrhosis
5	M	22	Bridging portal fibrosis with mononuclear cellular infiltrate
6	F	32	Micronodular cirrhosis with mononuclear cellular infiltrate
7	M	55	Lobular hepatitis and limiting plate mononuclear cellular infiltrate
8	M	29	Submassive hepatic necrosis
9	F	39	Chronic cholestatic hepatitis
10	M	30	Lobular hepatitis and limiting plate mononuclear cellular infiltrate
11	M	38	Micronodular cirrhosis with mononuclear cellular infiltrate
12	M	22	Micronodular cirrhosis with mononuclear cellular infiltrate
13	F	27	Lobular hepatitis and limiting plate mononuclear cellular infiltrate
14	M	33	Micronodular cirrhosis with mononuclear cellular infiltrate
15	F	20	Massive hepatic necrosis
16	F	40	Periportal inflammation and lobular cells cholestasis

During follow-up, 2 (6.1%) patients who were on D-penicillamine therapy demonstrated disease progression and a second drug was added to the treatment (zinc acetate). Combination therapy stabilized disease progression in both cases but did not improve the symptoms.

Liver transplantation was indicated in one patient who developed chronic progressive liver failure after 8 years of D-penicillamine treatment.

One asymptomatic subject diagnosed through genetic family screening remained without therapy and was receiving periodic clinical-radiological follow-up.

Outcomes

The mean duration of treatment with D-penicillamine was 129.9 $\pm$ 108.3 (5–468) months.

At the end of the follow-up, we evaluated whether hepatic features and neuropsychiatric signs had stabilized, improved or worsened. Six (18.2%) patients showed signs of improvement, and 20 (60.6%) patients remained clinically stable. Another six (18.2%) subjects presented a progressive disease with worsening of symptoms (4 patients with neurological disease and 2 patients with hepatic disease), although one of them had a significant alcoholic addiction and therefore poor treatment compliance. Three (9.1%) patients died, one of whom died due to the development of cancer (leukemia). Patients with chronic liver disease were periodically screened for hepatocellular carcinoma, and none of them developed it. The patient who had the liver transplantation has remained clinically stable for 4 years after the procedure and is still receiving follow up. The overall survival rate was of 90.1% at the end of the follow-up period.

Epidemiological and Social Features

Fourteen of the 26 subjects who were older than 25 years were married, and 8 (57.2%) of them had children. After the diagnosis, one female patient with predominantly neurological features had 8 miscarriages, and 2 others also had miscarriages before a successful pregnancy. In one patient, treatment with D-penicillamine was switched to zinc acetate during pregnancy, and no abnormalities were observed during the pregnancy or in the offspring.

Among the 33 patients older than 18 years, 14 (38.9%) have work skills and are employed, and 2 subjects have bachelor's degrees. Most of the subjects are underemployed. Two (6.1%) of the working-age subjects are retired because of limitations associated with WD. Three (8.3%) patients are younger than 18 years, and all of them are studying properly.

DISCUSSION

The results of the present study describe a historical cohort of 36 WD patients with an equal distribution between the genders. The mean age at diagnosis is similar to the ones described previously by other authors.[12-14] There are, however, reports of onset of symptoms on the extremes of age.[15,16] Thus, WD must be considered at all ages.

Characteristically, our study population is composed mainly of subjects with a European continental origin. This distribution reflects the European colonization of southern Brazil during the nineteenth century, particularly by Germans, Poles and Italians.[17] This distribution differs from the rest of the country, where a mixed continental origin is more common, mostly because of a high prevalence of native indigenous and African ancestors.[18]

Recent literature shows a progressive reduction of the gap between the initial symptom and the definitive diagnosis.[19] On the contrary, the mean gap in this study (27.5 months) still demonstrates a significantly long period of time for the correct diagnosis of WD to be established, even in the more recent decades of this 40-year analysis. Similar results (25.3 months) were observed in another study, and the authors state that because there are several different clinical presentations of WD, the analysis of the diagnostic findings underscores the difficulties in diagnosing WD.[20] The delay in the diagnosis is regrettable because the longer the disease progresses without treatment, the more likely that the damage to the liver and/or brain will be permanent.

The time lag from the appearance of the first symptoms to the diagnosis showed a great amount of variation, from 1 to 194 months. The neurological presentation was associated with a statistically significantly longer period of time from the onset of symptoms to the diagnosis than the hepatic presentation. Although there is not a consensus currently, several literature reports corroborate this finding.[10,20,21]

Although the pathogenic defect of WD is located in the hepatobiliary system, the consequences of copper accumulation may be detected in several organs and tissues.[8] The most important clinical manifestations of copper overload are hepatic (45%–58%), neuropsychiatric (30%–33%) or a combination of both.[20,22,23] Clinical manifestations among the 36 cohort patients are similar to these proportions.

Usually, hepatic disease occurs between the first and second decades of life, whereas neurological symptoms develop later.[10] In fact, the study conducted by Merle *et al.* in 2007 demonstrated that patients with mainly hepatic symptoms showed an overall earlier onset of symptoms (mean age: 15.5 years) than patients with predominantly neurological symptoms (mean age: 20.2 years).[20] Our results, probably restricted by the small number of subjects, show no differences in the age of the onset of the symptoms among patients with exclusively neuropsychiatric disease, exclusively hepatic disease or mixed presentation.

Most authors agree that the majority of WD patients have signs of hepatic disease, and some authors believe that all patients have some degree of hepatic dysfunction.[22] In fact, the manifestations of hepatic disease may vary widely, from nonspecific hepatomegaly to severe forms of liver failure.[21] As demonstrated in this study, the development of progressive chronic liver disease occurs most frequently. In general, liver histological analysis results in nonspecific multivariable features common to other chronic liver diseases.[24] The data from the present study corroborate these findings.

Kayser-Fleischer rings were detected in 55.6% of the subjects, with a higher frequency among those patients with exclusively neuropsychiatric symptoms (77.8%). Previous reports described the presence of the Kayser-Fleischer rings in WD patients with a frequency range of 66.3% to 95%.[20,25,26] In theory, this sign is observed in virtually all patients with neurological disease, although studies have reported lower frequencies, such as 85%.[20] The relatively low frequency of Kayser-Fleischer rings observed among our patients with neuropsychiatric symptoms may be underestimated because of the variation in the slit lamp exam's quality during the 40-year period that we retrospectively analyzed but may also underscore the difficulties in diagnosing WD.

At presentation, neuropsychiatric signs were the exclusive features in 25% of the WD patients. About 40%–50% of patients with WD have neurological or behavioral symptoms at the time of diagnosis.[10] Advances in neuroimaging have improved our understanding of the pathophysiology of WD. Besides the ''face of giant panda'' sign, hyperintensities in tectal plate and the central pons as well as the simultaneous involvement of the basal ganglia, thalamus and brainstem are almost pathognomonic of WD.[28] MRI changes tend to be more severe and widespread in patients with neurological disease,[29] with a strong correlation with disease severity scores.[30]

Lifelong treatment is required for WD. The best therapeutic approach remains controversial, and there is no universally accepted regimen.[10] In this cohort, we describe the analysis of more than 10 years of follow up during WD treatment.

The majority of our patients were treated with D-penicillamine. The choice for zinc salts was made in asymptomatic patients, during pregnancy or to potentially avoid further acute neurological deterioration. Although controversial, recent data have suggested that D-penicillamine should not be the drug of choice for patients with neurological features because of the drug's potential to induce the development or worsening of neurological symptoms.[20] When signs of treatment failure occurred, a second drug was added to the therapy. Of note, none of the patients in this cohort were on trientine treatment because trientine treatment is not available through Brazilian public health assistance.

With medical treatment and liver transplants, 72.2% of the patients stabilized or improved. The study of Merle et al. described similar results, and 76.1% of their analyzed patients experienced a stable or improved course of disease[20], highlighting not only the importance of early diagnosis but also compliance to therapy.

The development of new symptoms or the progression of preexisting symptoms was more often recorded for patients with neuropsychiatric symptoms (4/6 subjects) than for patients with hepatic symptoms (2/6 subjects). Not unexpectedly, the deterioration of the disease in its various forms was detected almost invariably in noncompliant patients. Clinical deterioration, despite the appropriate treatment, is highly dependent on the compliance of how early therapy is started and on the individual's response to therapy.[31]

Three patients died during follow-up; one patient died because of the development of a blood malignancy (leukemia). Despite the description of a common mechanism of copper accumulation in WD and cancer, hepatocellular carcinoma and other tumors of the liver are extremely rare in patients with WD. Although the causes are dramatically different, cancer shares a similar defect in copper homeostasis with WD; therefore, treatments like copper chelation therapy have been used against cancer.[32]

There are only a few reports addressing fertility and the outcome of pregnancy in WD.[33,34] As observed here, recurrent miscarriages are common, especially in women with untreated WD. However, successful pregnancies and full-term delivery may occur.[34] Data on quality of life and social features are scarce in the literature.[35] Because WD has a long overall survival with adequate treatment, these features need to be analyzed. The present results demonstrate that besides a positive life expectancy, WD patients can achieve an adequate pattern of quality of life, work skills and social inclusion.

To conclude, this study is the first clinical description of a cohort of WD patients with mainly European continental origin who live in southern Brazil. Therefore, the phenotypic features detailed in this study may contribute to our knowledge about the regional distribution of the disease in a country with such a highly multiracial population. As demonstrated here, WD is treatable if correctly diagnosed, and an adequate quality of life can be achieved with treatment, resulting in a long overall survival. Because there is no consensus therapy, treatment should be individualized, and careful follow-up is mandatory.

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