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Worsening of Wilson Disease following Penicillamine Therapy

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Key Words
Chronic liver disease · Copper · Leukopenia · Pancytopenia · Penicillamine · Wilson disease · Worsening of Wilson disease

Abstract
Background: Penicillamine is a standard therapy for Wilson disease (WD) but some patients have paradoxical worsening. Predictors of such deterioration have not been evaluated. This study documents frequency and predictors of deterioration following treatment in WD. ***Methods:*** 59 consecutive patients with neurologic WD and 4 asymptomatic siblings were prospectively evaluated. Their clinical, laboratory, ultrasound abdomen and cranial MRI findings with and without worsening were compared. Patients were treated with oral penicillamine and/or zinc and followed up at 1, 3 and 6 months or earlier if needed. Deterioration was defined by >10% worsening in baseline Burke-Fahn-Marsden score or appearance of new neurological sign. ***Results:*** Patients' median age was 13 years and 13 were females. 19 patients (30.2%) worsened following treatment; 10 within 1 month, 7 in 1–3 months, and 2 after 3 months of treatment. Deterioration was associated with drooling, leukopenia, thrombocytopenia, splenomegaly and evidence of chronic liver disease. None of the asymptomatic patients following zinc therapy deteriorated. ***Conclusions:*** In the deteriorating group, withdrawal of penicillamine resulted in improvement/stabilization in 11 patients, 2 improved by trientine therapy and 4 continued to deteriorate till 3 months. 30.2% patients with WD deteriorated following penicillamine, especially those with chronic liver disease, leukopenia and thrombocytopenia.

Introduction

Wilson disease (WD) is an autosomal recessive disorder due to mutation in ATP7B gene localized to chromosome 13q14.3 [1]. The worldwide prevalence of WD is 1 in 30,000 to 1 in 100,000 [2]. The encoding protein by ATP7B gene is essential for copper transport and elimination of excess copper in the body, therefore ATP7B gene mutation results in excessive copper accumulation in the liver, eye, brain, kidney, heart and other organs. Since the discovery of penicillamine in 1954, it is the standard decoppering therapy used in WD. Penicillamine increases urinary excretion of copper resulting in negative copper balance which is the goal of effective therapy of WD. Unfortunately, penicillamine has been reported to result in deterioration in up to 50% patients with WD and half of these patients do not improve to baseline even after discontinuation of the drug [3]. The other agents used in the treatment of WD are trientine, ammonium tetrathiomolybdate and zinc. Penicillamine and trientine both are

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chelating agents and act by binding copper in the body resulting in increase in urinary excretion. Ammonium tetrathiomolybdate acts by forming a tripartite complex with copper and protein either in the intestinal lumen preventing copper absorption or in the circulation making the copper unavailable for cellular uptake [4–8]. Zinc acts through blocking the carrier in the intestinal epithelial cells which prevents copper transport. The management of patients with neurological WD is controversial and the choice of treatment depends on physicians' preference and availability and affordability of the drug. Penicillamine is the only copper-chelating drug available in India. In this communication, we report the response to penicillamine treatment in patients with WD and predictors of worsening in them.

Material and Methods

This study was approved by the Institutional Ethics Committee of SGPGIMS, Lucknow, India, and all the patients or their first-degree relatives gave informed consent.

Consecutive patients with neurological WD and 4 asymptomatic siblings with WD attending the neurology service of a tertiary care center during 2006–2011 were included. The diagnosis of WD was based on clinical symptoms and signs, reduced serum ceruloplasmin <20 mg/dl and presence of Kayser-Fleischer ring on slitlamp examination. The patients were subjected to a detailed clinical examination including pedigree charting. Presence of osteoarticular deformity, bone pain, jaundice, hepatosplenomegaly, anemia and renal dysfunction were noted. Cognitive functions were assessed by Mini Mental State Examination (MMSE). Various movement disorders such as dystonia, parkinsonian features, chorea, athetosis, myoclonus and tremor were recorded and videotaped. The severity of dystonia was assessed using Burke-Fahn-Marsden (BFM) score [9]. The severity of other movement disorders was graded on a 0–IV scale [10, 11]. Muscle power, tone and reflexes were noted. Sensation of joint position and pinprick were tested. The severity of neurological WD was based on activities of daily living (ADL) and sum score of 5 signs which include dysarthria, tremor, ataxia, rigidity/bradykinesia, chorea/dystonia on a 0–3 score for each in which 0 is none and 3 is severe. The severity of neurologic WD was graded into grade 0: absent, grade I (mild, sum score 1), grade II (moderate, sum score of 2–7 and the patient is independent for ADL) and grade III (severe, sum score >7, dependent for ADL) [12].

Investigations

Blood counts, hemoglobin, erythrocyte sedimentation rate, coagulation profile, fasting and postprandial blood sugar, serum creatinine, bilirubin, transaminase, albumin, calcium, phosphorous, alkaline phosphatase, sodium and potassium were done in all the patients. Serum copper, ceruloplasmin, and 24 h urinary copper were also measured. Cranial MRI was carried out using 1.5-T unit from GE Signal Medical Systems, Milwaukee, Wisc., USA. T1, T2 and FLAIR images were obtained in axial and sagittal sections. The abnormal signal changes and their locations were recorded. The number of MRI lesions was considered as MRI load of WD in an individual patient.

Treatment and Follow-Up

The patients were treated with penicillamine, zinc or both. The patients were followed up at 1, 3 and 6 months or at the time of worsening. During the follow-up, appearance of new symptoms and signs, BFM score, the severity of neurologic disease and blood counts were done. Liver and kidney functions were evaluated if needed. The patient was considered to worsen if there was at least 10% deterioration in the baseline BFM score or appearance of new neurologic symptoms and signs.

Statistical Analysis

For statistical analysis the patients were grouped into worsening and no worsening groups (improved and stabilized were grouped as no worsening group). The baseline demographic, clinical, laboratory and MRI findings were compared between the two groups. The categorical variables were compared using Fisher's exact and the continuous variables by independent t test or Mann-Whitney U test. The best set of predictors of worsening was derived by multivariate logistic regression analysis using the variable having a p value of <0.10 on univariate analysis. The variable was considered significant if the two-tailed p value was <0.05. The statistical analysis was done using SPSS version 16.

Results

There were 63 patients with WD from 56 families; 59 had neurologic WD and 4 asymptomatic siblings were diagnosed during screening of family members. The median age of onset of disease was 13 (4–41) years and 13 were females. 27 (42.9%) patients manifested in the first decade, 30 (47.6%) in the second decade, and 6 (9.5%) in the third decade of their life. The median delay from the onset of symptoms to reporting was 18 (1–156) months. The first presenting symptoms was neurological in 47 (74.6%), hepatic dysfunction in 6 (9.5%), psychiatric in 5 (7.9%), and skeletal in 1 (1.7%) patient; the remaining 4 (6.3%) were asymptomatic. All the 59 symptomatic patients had movement disorders. Walking difficulty was present in 50, drooling in 45, sleep disturbance in 11, behavioral abnormality in 25, and seizures in 10 patients. History of jaundice was present in 28, and 26 of them had splenomegaly. On examination, bone and joint deformity was present in 7 patients and included genu valgum in 4 and genu varum in 3 patients. MMSE was abnormal in 16 out of 45 patients in whom it was tested. Cerebellar signs were present in 6 and tendon reflexes were exaggerated in 39 patients. Isolated dystonia was present in 14, tremor in 2, and dystonia with other movement disorders in 43 patients (tremor 21, chorea 8, tremor and chorea 5, chorea

and myoclonus 5, choreoathetosis 2, and myoclonus 2). Oromandibular dystonia was the commonest being present in all 57 symptomatic neurologic WD patients. Limbs dystonia was present in 51, neck dystonia in 37, and trunk dystonia in 33 patients. The baseline severity of WD was of grade III in 28, grade II in 26, grade I in 5, and grade 0 in 4 patients. The BFM score was 42.75 ± 28.93.

Investigations

The median hemoglobin level was 11.9 (9.5–15.6) g/dl and 33 (52.4%) patients were anemic. Leukopenia was present in 7 (11.1%) and thrombocytopenia in 49 (77.8%) patients. Reticulocyte count was done in 23 patients and the reticulocyte production index was <2.5 in them suggesting bone marrow suppression. Serum creatinine was high in 1, bilirubin in 3, SGOT in 38, SGPT in 25, SGOT and/or SGPT in 43, and alkaline phosphatase in 57 patients. Serum albumin was low in 15 patients and calcium in 13 patients. MRI was done in 52 patients and was abnormal in 51.

Treatment and Follow-Up

Twenty-four patients were treated with penicillamine, 9 with zinc acetate and 30 with both penicillamine and zinc acetate. They were also prescribed anticholinergic and benzodiazepine for their dystonia. The median follow-up was 8 (3–156) months. 63 patients were followed up for 6 months and 19 (30.2%) of them deteriorated. The worsening in the clinical picture occurred at different time after starting therapy. 10 patients deteriorated within 1 month, 7 within 1–3 months, and 2 after 3 months of treatment (fig. 1). At the time of deterioration, the median dose of penicillamine was 500 (range 125–1,000) mg and zinc was 100 (range 40–150) mg. All the 19 patients deteriorated at least by 10% in the baseline BFM score; 7 of these patients had grade I deterioration in the severity grading and in 2 patients additional neurological signs and symptoms appeared. Two of these patients also had behavioral deterioration; 1 developed obsessive-compulsive behavior and the other developed irritability and aggression. The laboratory worsening however was seen in the patients with and without neurological worsening (p = 0.37); 7 patients (5 hematological, 4 hepatic and 1 renal) with neurological worsening and 11 patients without had laboratory worsening (8 hematological, 7 hepatic).

Predictors of Worsening

On univariate analysis, drooling (p = 0.007), leukopenia (p = 0.002), thrombocytopenia (p = 0.03), splenomegaly (p = 0.04) and evidence of chronic liver disease (CLD) (p = 0.04) were significantly related to worsening. The other parameters such as age (16.5 ± 8.1 vs. 14.8 ± 6.1 years, p = 0.37) gender (p = 1.00), presenting symptoms (p = 0.31), duration of illness (p = 0.81), MMSE score (p = 0.98), severity of WD (p = 0.87), BFM score (p = 0.39), anemia (p = 0.27), serum albumin (p = 0.98), bilirubin (p = 0.65), elevated liver enzymes (p = 1.00) and creatinine (p = 0.05) were not related to worsening. The dose of penicillamine (434.2 ± 247.8 vs. 485.7 ± 232.4 mg, p = 0.45), serum copper (p = 0.61) and 24 h urinary copper exertion (p = 0.51) were also not related to worsening. The location and number of MRI lesions were also not related to worsening (tables 1, 2). On multivariate regres-

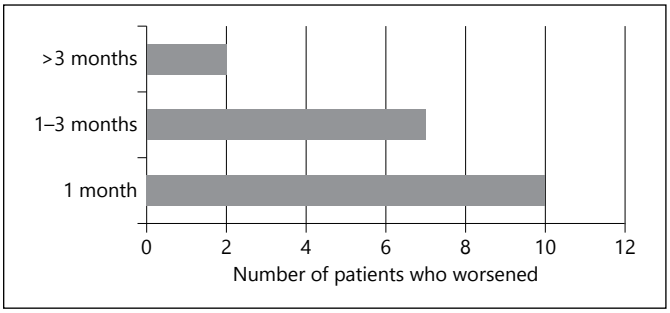

Fig. 1. Number of patients who worsened at a different time interval following treatment.

Table 1. Demographic and clinical variables of patients with WD having worsening after penicillamine compared to those without

	Worsening (n = 19)	No-worsening (n = 44)	p value
Age, years	16.5±8.1	14.8±6.1	0.37
Females	4	9	1.00
Duration of illness, months	31.5±39.8	34.1±38.5	0.81
Movement disorder	19	40	0.31
Walking difficulty	18	32	0.09
Drooling of saliva	18	27	0.007
Seizure	1	9	0.26
Family history	4	20	0.09
History of jaundice	12	16	0.09
Splenomegaly	12	14	0.03
MMSE score	25.3±4.6	25.3±6.1	0.98
Rigidity	16	29	0.22
Oromandibular dystonia	19	38	0.17
Limbs dystonia	18	33	0.09
Tremor	10	18	0.42
Myoclonus	4	3	0.18
Chorea	9	11	0.14
Athetosis	0	2	1.00
Severity score	11.5±5.8	10.2±7.2	0.47
BFM score	47.5±21.7	40.7±31.5	0.39

Table 2. Laboratory and MRI findings in patients with WD having worsening after penicillamine compared to those without

	Worsening (n = 19)	No-worsening (n = 44)	p value
Hemoglobin, g/dl	11.6±1.2	12.0±1.2	0.21
Leukocytes/mm^3	5,157±2,194	6,615±2,587	0.036
Leukopenia	6	1	0.002
Platelets/mm^3	98.20±44.8	140.9±79.7	0.032
S. creatinine, mg/dl	0.80±0.18	0.94±0.28	0.05
S. protein, g/dl	6.9±0.97	7.1±0.75	0.49
S. albumin, g/dl	3.76±0.67	3.75±0.58	0.98
S. bilirubin, mg/dl	0.73±0.34	0.69±0.34	0.65
↑ Liver enzymes	13	30	1.00
S. calcium, mg/dl	9.18±1.08	9.15±1.08	0.91
S. ALP, U/l	382.2±197.5	355.8±191.3	0.63
Penicillamine, mg/day	434.2±247.8	485.7±232.4	0.45
Zinc, mg/dl	125.0±100.7	123.9±83.85	0.97
Urine copper, μg/day	168.4±82.2	278.95±398.2	0.51
Serum copper, μg/dl	80.7±8.49	76.68±16.7	0.61
CLD on USA	12	14	0.03
Abnormal MRI	16	35	0.78
Thalamic	12	17	0.08
Putamen	15	30	0.42
Caudate	10	24	0.76
Globus pallidus	10	19	0.56
Brainstem	8	21	0.76
Cerebellar	0	2	1.00
SCWM	3	10	0.73
Cortical	3	7	1.00
Number of MRI lesion	7.4±3.0	7.2±2.8	0.98

ALP = Alkaline phosphatase; CLD = chronic liver disease; SCWM = subcortical white matter; USA = ultrasound abdomen.

sion analysis, none of the variables were significantly related to worsening. Urinary copper and serum copper were repeated in 20 patients. Serum copper in the patients with worsening and non-worsening group (80.7 ± 8.5 vs. 76.7 ± 16.7 μg/dl, p = 0.61) was not significantly different. 24 h urinary excretion of copper was insignificantly reduced in the patients who deteriorated compared to those who did not (168.4 ± 82.2 vs. 278.9 ± 398.2 μg, p = 0.51).

Outcome

Patients were followed up for a median of 8 (3–156) months. 44 patients did not worsen on treatment, of whom 16 were improved (grade I in 15, grade II in 1) and 28 remained in the same condition; they continued the same treatment. In the patients who deteriorated, penicillamine was withdrawn and zinc was continued in a median dose of 100 (range 100–150) mg daily. Trientine was prescribed in 2 patients and both improved; 1 had complete recovery from grade III at 6 months and the other improved by grade I. In the remaining 17 patients, 5 improved on zinc, 6 stabilized on zinc, and 4 kept on deteriorating till 3 months of withdrawal of penicillamine; 2 patients were lost to follow-up.

Discussion

In the present study, 30.2% patients with WD worsened following treatment, especially those having evidence of CLD, leukopenia and thrombocytopenia. The deterioration in these patients was likely due to penicillamine, especially in those 13 patients who stabilized or improved after stopping penicillamine in spite of continued zinc therapy. 4 asymptomatic patients who were only on zinc therapy did not have worsening. In a retrospective review of 27 patients with neurologic WD, 13 had shown initial deterioration usually within 2–6 weeks of penicillamine therapy. 6 of these 13 patients who deteriorated never improved to baseline even after discontinuation of penicillamine [3]. In another study, initial neurological deterioration was observed in as high as 75% of patients following penicillamine whereas none deteriorated following zinc [13]. Many other studies, however, did not report deterioration following penicillamine [14–19]. In a systematic review of penicillamine and zinc therapy in WD, overall unfavorable outcome was reported in 14.6% patients; 9.6% deteriorated, 3.5% died and 1.5% remained unchanged. Following zinc therapy, unfavorable outcome was observed in 5.4% patients only, 1.5% deteriorated, 1.2% died, and 1.2% remained unchanged. None of the asymptomatic patients deteriorated following penicillamine or zinc therapy [20]. All 4 asymptomatic patients in our study also did not have deterioration after zinc therapy. One third of our patients deteriorated and 50% of them deteriorated within 1 month of treatment. Only 2 patients deteriorated after 3 months of penicillamine therapy. Our results are somewhat similar to those of Brewer et al. [3] and Medici et al. [13] though the frequency of deterioration was lower than their studies. Our results are based on a large sample size, relatively homogenous sample (all neurological WD except 4) and we have used a quantitative neurological score (BFM) for defining worsening.

Chelating agents decrease free copper concentration rapidly by enhancing excretion of copper through kidney. On the other hand, zinc reduces intestinal absorption of copper which results in a mild negative copper balance. For excretion of mobilized copper by chelating

agents normal renal functions are essential. In our study, an insignificantly higher serum copper and lower 24 h urinary copper suggest poorer urinary excretion which may be due to subtle renal dysfunction. After chelating therapy an increase in serum copper in these patients may result in systemic and neurological toxicity and might explain the frequent deterioration following penicillamine therapy. Detailed renal function tests and copper levels before and after the chelating agent may throw some light in this direction, however we have not studied detailed renal function tests such as glomerular filtration rate and tubular function tests. Conversely D-penicillamine and trientine are used to chelate copper in the WD patients with fulminant liver failure, hemolytic anemia, or both [21, 22]. None of the studies in the available literature have evaluated the predictors of deterioration following treatment. Association of leukopenia, thrombocytopenia, and CLD suggest widespread copper toxicity. Leukopenia, thrombocytopenia and anemia in WD may be due to hypersplenism or bone marrow suppression. In our study, the spleen was enlarged in 57.9% in the deterioration group and 34% in the non-deterioration group. The number of CLDs was high in the deterioration group (57.9 vs. 34.0%) compared to no deterioration. Evidence of hemolysis (raised LDH and bilirubin) was present in 1 patient only, pancytopenia in 9.5% and LDH was raised up to two times in 43.5% patients (10/23). Reticulocyte production index was <2.5 in all 23 anemic patients in whom it was measured suggesting bone marrow suppression. Bone marrow suppression in WD may be due to copper deposition or a side effect of penicillamine. We have used baseline data for predicting the worsening, therefore penicillamine induced bone marrow is unlikely.

Two thirds of our patients who deteriorated improved or stabilized after discontinuation of penicillamine. Zinc was continued in these patients. Two of our patients improved on trientine which is also a chelating agent and is officially approved by the FDA as an alternative to penicillamine in patients intolerant to penicillamine. The exact mechanism of improvement in these 2 patients on trientine, which is also a chelating agent, is not well understood. In a randomized double-blind study, trientine was compared with ammonium tetrathiomolybdate in patients with neurological WD. It was found that tetrathiomolybdate was superior to trientine. In this study, 6 out of 23 (26%) patients on trientine deteriorated [23, 24]. The patients who deteriorated on penicillamine are a great challenge and the safety and efficacy of alternative drugs including trientine needs to be explored. Currently our patients are maintained on zinc because of non-availability and non-affordability of alternative therapeutic agents.

It can be concluded that one third of neurological WD patients worsen on penicillamine therapy, especially if they have chronic liver disease, leukopenia or thrombocytopenia. In view of non-availability and high cost of trientine in India as well as improvement in 70% neurologic WD following penicillamine, we feel that WD patients may be started with low-dose penicillamine which should be escalated slowly with close monitoring till an alternative safe drug is available.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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