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Hepatology

Relapse following withdrawal of D-penicillamine from combination (D-penicillamine + zinc) therapy in hepatic Wilson disease

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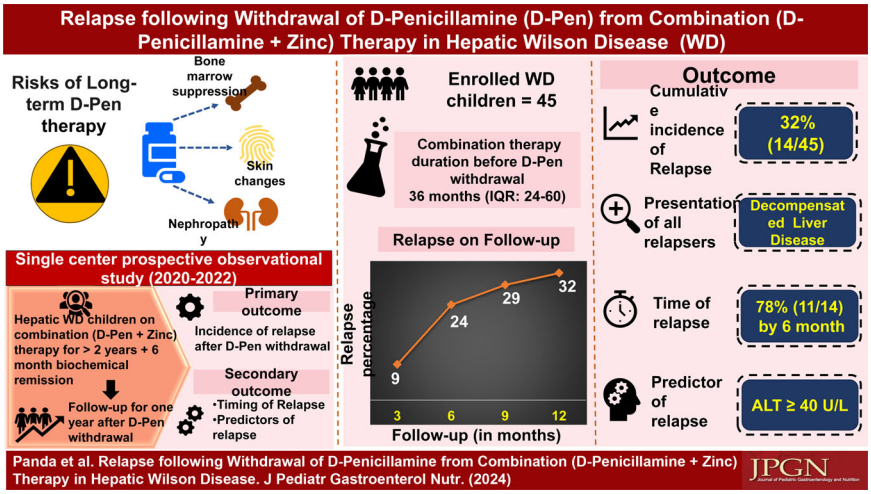
Abstract

Objectives: Long-term D-penicillamine (D-pen) therapy in Wilson disease (WD) has numerous adverse effects which advocates its withdrawal, but with an inherent risk of relapse. This prospective observational study was conducted with the objective of evaluating incidence of relapse following withdrawal of D-pen from combination (D-pen + zinc) therapy in maintenance phase of previously symptomatic hepatic WD.

Methods: Hepatic WD patients <18 years of age and on combination therapy for >2 years with 6 months of biochemical remission were included. Biochemical remission was defined as achievement of (i) aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤1.5 times upper limit of normal (ULN), (ii) serum albumin >3.5 g/dL, international normalized ratio (INR) <1.5 and (iii) 24-h urinary copper excretion (UCE) <500 mcg/day, nonceruloplasmin-bound-copper (NCC) <15 mcg/dL. After D-pen withdrawal, monthly liver function test (LFT) and INR and 3 monthly UCE and NCC were done till 1 year or relapse (elevation of AST/ALT/both >2 times ULN or total bilirubin >2 mg/dL), whichever occurred earlier.

Results: Forty-five patients enrolled with median combination therapy duration of 36 months. Sixty percent of them had their index presentation as decompensated cirrhosis. Fourteen patients (31.8%) relapsed (cumulative incidence: 4 at 3 months, 11 at 6 months, and 14 at 12 months after D-pen discontinuation). All relapsers had index presentation as decompensated cirrhosis. On Cox-regression, ALT at D-pen withdrawal was an independent predictor of relapse (hazard ratio [HR]: 1.077, 95% confidence interval [CI]: 1.014–1.145, $p = 0.017$) with area under the receiver operating characteristic (AUROC) of 0.860. ALT ≥40 U/L predicted risk of relapse with 85.7% sensitivity, 70.9% specificity.

Conclusion: Incidence of relapse after withdrawal of D-pen from combination therapy is 31.8% in hepatic WD. ALT ≥40 U/L, at the time of D-pen stoppage, predicts future relapse.



KEYWORDS

children, D-pencillamine, relapse, Wilson disease, zinc

1 | INTRODUCTION

Wilson disease (WD), is an inherited disorder of copper metabolism, leading to its accumulation in liver, brain, cornea, kidney, and so on.[1,2] Although it has been five decades since first introduction of D-penicillamine (D-pen) as a chelating agent for WD, there is still significant controversy regarding optimal medical therapy, especially in maintenance phase treatment of hepatic WD. These patients require lifelong treatment and long-term D-pen therapy has potentially serious side effects like bone-marrow suppression causing cytopenias, nephropathy, lupus-like syndrome, skin changes, and so on,[3–9] which leads to treatment discontinuation in approximately 10%–30% as reported in various WD cohorts.[10–14] Zinc (Zn), an intestinal inhibitor of copper absorption, has minimal side effects compared to D-pen.[15–19] Furthermore, D-pen is three times costlier than Zn, making it cost-prohibitive in resource-limited settings. Zn monotherapy has been extensively studied and recommended as first-line treatment for presymptomatic WD[20–23] and has proven to be highly effective. However, its role in management of hepatic WD patients, especially as monotherapy in maintenance phase after initial decoppering with a chelator, is a matter of debate. Very little scientific literature is available regarding clinical course and outcome of patients who have been shifted from combination therapy/chelator monotherapy to Zn monotherapy. Moreover, most of these studies are retrospective and undertaken predominantly among adult subjects.[24–27] Hence, this study was conducted to evaluate the incidence of relapse after withdrawal of D-pen from combination (D-pen + Zn) therapy in maintenance phase treatment of pediatric hepatic WD.

What is Known

- Nearly 32% of hepatic WD children relapse after withdrawal of D-pen from combination therapy. Majority of relapses occur within 6 months of D-pen discontinuation.
- WD patients with index presentation as decompensated cirrhosis are at risk for relapse.
- Alanine aminotransferase (ALT) ≥40 U/L at the time of D-pen withdrawal is a predictor for future relapse.

What is New

- Long-term D-penicillamine (D-pen) therapy in Wilson disease (WD) has serious side effects which advocates its withdrawal, but with an inherent risk of relapse.
- Relapse rate after withdrawal of D-pen from combination (D-pen + zinc) therapy in maintenance phase of symptomatic hepatic WD has not been studied previously.

2 | METHODS

This prospective observational study was conducted in the Department of Pediatric Hepatology at Institute of Liver and Biliary Sciences (ILBS), New Delhi from September 2020 to September 2022. The study was approved by the Institutional Ethics Committee (approval no: IEC/2020/76/NA05). Our primary objective was to study the incidence of

relapse following withdrawal of D-pen from the combination (D-pen + Zn) therapy after 2 years of treatment and 6 months of biochemical remission in formerly symptomatic hepatic WD patients. Our secondary objectives were to study the timing of relapse after such withdrawal and to identify clinical/biochemical predictors for relapse.

We included predominantly hepatic WD patients of age <18 years, who were on combination therapy for >2 years; and had been in biochemical remission for at least 6 months with a stable compensated chronic liver disease (cCLD) status. For our study purpose, biochemical remission had been defined as fulfillment of all the following criteria (i) aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤1.5 times upper limit of normal (ULN), (ii) serum albumin >3.5 g/dL and international normalized ratio (INR) <1.5, (iii) 24-h urinary copper excretion (UCE) <500 mcg/day, and (iv) nonceruloplasmin-bound copper (NCC) <15 mcg/dL. For both AST and ALT, 40 U/L was considered arbitrarily as ULN. WD patients who were either presymptomatic (detected on sibling screening) or in a decompensated state at the time of screening for enrollment into study had been excluded. Also, WD patients with another coexisting liver disease (nonalcoholic fatty liver disease/chronic hepatitis-B/hepatitis-C) or unwilling for close follow-up were excluded. D-pen was stopped at once without any tapering and Zn was continued in all patients. Zinc-acetate was prescribed as per the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) position paper.[22]

Baseline (at time of enrollment into study) evaluation included a detailed clinical status assessment and biochemical analysis that is, liver function test (LFT), INR, complete haemogram, urine routine microscopy, UCE, and NCC. 24-h urine copper and serum copper analysis were performed using inductively coupled plasma mass spectrometry.[28,29] NCC was calculated using equation: NCC = serum total copper − 3 × serum ceruloplasmin. All study participants were followed up every month with LFT and INR till 1 year of D-pen stoppage or relapse whichever occurred earlier. Apart from monthly LFT and INR, each of the included patients was called up for an outpatient visit at 3-, 6-, 9-, and 12-month/relapse whichever occurred earlier for evaluation of clinical status, drug compliance as well as complete biochemical assessment including copper studies. Relapse was defined as an elevation of AST/ALT, or both, >2 times ULN (i.e., >80 U/L) or serum total bilirubin >2 mg/dL, on two separate occasions at least 2 weeks apart. After exclusion of other attributable causes of LFT derangement (viral infections/drug-induced liver injury/nonalcoholic fatty liver disease), D-pen was restarted immediately in relapsers and Zn was continued.

As it was a pilot study, all pediatric WD patients fulfilling inclusion criteria had been enrolled.

Statistical analyzes were performed using SPSS version 28.0 (IBM SPSS, IBM Corporation). Non-parametric tests were used to compare between various groups. Wilcoxon Signed Rank test was used for comparison between paired samples. Mann–Whitney *U*-test was used for independent sample comparison. Multiple group comparisons were done by using Friedman's two-way analysis of variance (ANOVA). Cox-regression model was used to identify predictors of relapse after D-pen withdrawal. Area under receiver operating characteristic curve (AUROC) was calculated for the independent predictors of relapse and best cut-off was determined by Youden index.

3 | RESULTS

Out of 94 pediatric WD patients screened, 45 patients (male: 24 [53.3%]) fulfilled inclusion criteria and hence enrolled after parental consent. Among them, 60% had index presentation as decompensated cirrhosis (22 [48.8%]: decompensated chronic liver disease [dCLD] and 5 [11.1%]: acute on chronic liver failure [ACLF]) whereas rest 18 (40%) had initial manifestation as cCLD (hepatomegaly/transaminitis). Median age at diagnosis and enrollment into study were 9.8 years (interquartile range [IQR]: 8.2–11.5) and 13.6 years (IQR: 11.7–16.6) respectively. The median BMI percentile was 33.0 (IQR: 6.0–67.0). Although all enrolled patients were cases with predominant hepatic involvement, 12 (26.7%) had associated neurological manifestations. All had good compliance with combination therapy and received treatment for a median 36 months (IQR: 24–60). Serum albumin and AST/ALT were normalized 7 months (IQR: 4.8–11.0) and 12 months (IQR: 7.5–14.5) after initiation of combination therapy respectively. UCE has come down to below 500 mcg/day within a median 1-year period of starting combination treatment. The last recorded dose of D-pen at study enrollment was 10.3 mg/kg/day (IQR: 7.0–14.4). Before withdrawal of D-pen, cohort had normal transaminases for a median duration of 22 months (IQR: 13.5–45.5). Baseline (i.e., at the time of enrollment into study) laboratory parameters are tabulated in SDC-S1.

Follow-up of enrolled patients: Except for one, all the other participants have completed 12 months of follow-up. This particular child expired from a nonliver-related cause (head trauma) after completing 9-months of follow-up. All the patients tolerated Zn monotherapy with minimal incidence of adverse reactions such as occasional epigastric discomfort and vomiting reported by four patients (8.8%). One patient with combined hepatic and neurological symptoms had intermittently missed doses of Zn, attributed to his behavioral issues. However, overall compliance of the cohort was good.

Incidence of relapse after withdrawal of D-pen: 13 out of 45 patients had an elevation of AST or ALT >2 times ULN with one patient having an additional increase in serum total/direct bilirubin to 3.5/2.9 mg/dL and these abnormal results were noted on two separate occasions at least 2 weeks apart. One patient developed uncorrectable coagulopathy at 1 month with a peak INR of 2.44 without accompanying elevation of AST/ALT or bilirubin and was considered as a case of relapse. Hence, a total of 14 relapses occurred among the 44 patients completing all the follow-ups till 1 year (31.8%) (excluding the one who expired after 9 months of follow-up). The time of relapse for these 14 patients after discontinuation of D-pen was as follows (one patient each at 1 and 2 months, two patients at 3 months, one patient at 4 months, six patients at 6 months, two patients at 9 months, and one patient at 12 months). Hence, cumulatively, the incidence of relapses after 3, 6, 9, and 12 months of D-pen stoppage were 4/45 (8.8%), 11/45 (24.4%), 13/45 (28.8%), and 14/44 (31.8%) respectively (Figure 1 and SDC-S2).

Change in biochemical parameters on follow-up: A statistically significant increase in AST, ALT, and NCC was noted at relapse in comparison to baseline (Table 1). The median AST (U/L) increased from a baseline value of 37.9 (IQR: 34.7–50.2) to 85 (IQR: 56.5–108.7) at relapse. Likewise, ALT (U/L) also showed a significant elevation (approximately three times) at relapse [120.5 [IQR: 87.7–185.5.5]) as compared to baseline (44.0 [IQR: 39–53.0]). However, the synthetic function (serum albumin and INR) was maintained. There was a statistically significant rise in NCC (mcg/dl) at relapse (11.9 [IQR: 8.0–19.3]) versus

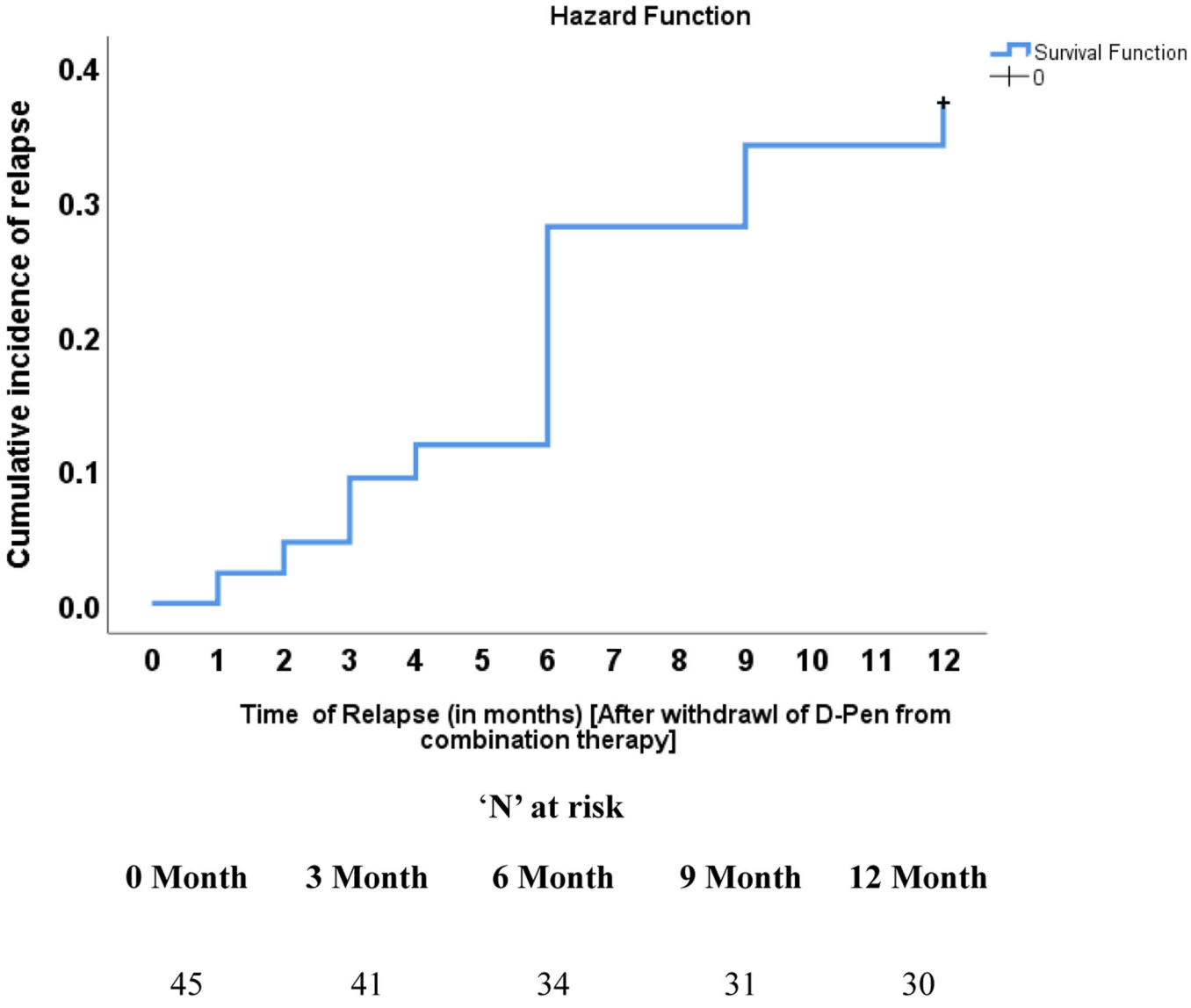


FIGURE 1 Kaplan–Meier Hazard curve showing cumulative incidence of relapse after withdrawal of D-penicillamine (D-pen) from combination therapy in the maintenance phase of hepatic Wilson disease (WD).

TABLE 1 Relative Change in biochemical parameters of relapsers ($n = 14$) at relapse in comparison to baseline (at study enrollment).

Parameters	Baseline	Relapse	*p*-Value
AST (U/L)	37.9 (34.7–50.2)	85 (56.5–108.7)	0.003
ALT (U/L)	44.0 (39.0–53.0)	120.5 (87.7–185.5)	0.020
Total bilirubin (mg/dL)	0.53 (0.42–0.91)	0.72 (0.43–1.06)	0.317
Albumin (g/dL)	4.34 (4.13–4.47)	4.40 (4.20–4.55)	0.317
INR	1.18 (1.08–1.26)	1.25 (1.07–1.34)	0.480
UCE (mcg/day)	260.0 (125.5–447.5)	414.6 (164.7–520.0)	0.363
NCC (mcg/dL)	−0.3 (−1.8 to 4.9)	11.9 (8.0–19.3)	0.034
Hemoglobin (g/dL)	12.1 (11.9–13.0)	12.4 (11.6–12.9)	0.348
Total leukocyte count (thousands/m^3)	5.7 (5.0–6.8)	5.4 (4.7–6.3)	0.612
Total platelet count (lakhs/mm^3)	157 (115–188)	163 (141–226)	0.352

Note: All values expressed as median (IQR).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; IQR, interquartile range; NCC, nonceruloplasmin bound; UCE, 24-h urine copper excretion.

baseline (−0.3 [IQR: −1.8 to 4.9]). UCE also increased to (414.6 [IQR: 164.7–520]) at relapse in comparison to baseline value of (260 [IQR: 125.5–447.5]) mcg/day. Although the increment in UCE at relapse was not statistically significant, median value of 414 mcg/day was far above the target range of 30–75 mcg/day considering patients were on Zn monotherapy.[22] Changes in biochemical parameters of nonrelapsers at various follow-ups in comparison to baseline can be referred from SDC-S3.

Predictors of relapse after withdrawal of D-pen from combination therapy in maintenance phase of hepatic WD (Table 2): On Cox-regression analysis including various enrollment clinical and biochemical parameters, five variables namely index hepatic manifestation (decompensated vs. compensated), system involved (pure hepatic vs. hepatic + neuro), dose of D-pen being administered at the time of withdrawal, time required for normalization of transaminases after index presentation and enrollment ALT were statistically significant on univariate analysis. All relapsers manifested initially in a decompensated state (either dCLD or ACLF) whereas almost half of nonrelapsers (48.4%) presented as cCLD. Likewise, all 14 relapsers were cases of pure hepatic WD without any evidence of neurological involvement. Relapsers were also taking higher doses of D-pen 11.9 (IQR: 9.5–16.5) mg/kg/day at the time of discontinuation, in comparison to nonrelapsers (8.9 [IQR: 6.0–13.8]). Furthermore, patients with relapse had taken a longer time for normalization of AST and ALT while on combination therapy (hazard ratio [HR]: 1.221, 95% confidence interval [CI]: 1.028–1.449; $p = 0.023$). Enrollment ALT at the time of D-pen cessation was significantly higher (HR: 1.071, 95% CI: 1.020–1.125; $p = 0.006$) in relapsers as compared to nonrelapsers. On multivariate analysis, enrollment ALT was the only independent predictor of relapse (HR: 1.077, 95% CI: 1.014–1.145, $p = 0.017$) and had AUROC of 0.860 (95% CI: 0.734–0.986) (Figure 2A). An ALT cut-off of ≥40 U/L predicted risk of relapse with 85.7% sensitivity, 70.9% specificity, and a prognostic accuracy of 75.5%. It had a very high negative predictive value (NPV) (91%); only 1 out of 11 children (9%) with baseline ALT <40 U/L had risk of developing a relapse (Figure 2B). Hence, the cumulative incidence of relapse at end of study duration was only 8.3% (2/24) in children with baseline ALT <40 U/L, in contrast to 57.1% (12/21) in those with ALT ≥40 U/L as shown in Kaplan–Meier hazard curve (Figure 2B).

Management of enrolled WD patients developing a relapse: All the relapsers were restarted on D-pen at 10 mg/kg/day and gradually built up to 20 mg/kg/day depending on the change in transaminases. After reintroduction of D-pen, AST and ALT started declining gradually in all of them within 2 months and normalized in 12 patients till the last follow-up.

4 | DISCUSSION

The treatment strategy for hepatic WD patients involves an initial decoppering phase with chelating agents (D-pen/trientine) aimed at decreasing body copper load to a subtoxic level followed by maintenance therapy to prevent reaccumulation of copper. Treatment options for maintenance phase include combination therapy using chelating agents at a lower dose with Zn, chelating agents alone, or Zn monotherapy.[20–23] There are no clear guidelines on the comparative efficacy of these drugs during maintenance phase. Long-term D-pen therapy is often associated with severe side effects, which results in treatment discontinuation in approximately 10%–30%.[10–14] There is

TABLE 2 Univariate and multivariate Cox regression analysis for risk predictive factors of relapse after withdrawal of D-penicillamine from combination therapy in the maintenance phase of hepatic Wilson disease.

			Univariate analysis		Multivariate analysis	
Variable	Relapsers ($n = 14$)	Nonrelapsers ($n = 31$)	HR (95% CI)	p-Value	Adjusted HR (95% CI)	p-Value
Age at study enrollment (years)	11.8 (10.6–15.0)	13.8 (12.4–16.9)	0.841 (0.683–1.036)	0.103		
Age at diagnosis[a] (years)	8.8 (7.0–10.3)	10.8 (8.5–12.0)	0.892 (0.748–1.064)	0.205		
Gender						
Male, *n* (%)	9 (64.3%)	15 (48.4%)	1.920 (0.523–7.049)	0.358		
Female, *n* (%)	5 (35.7%)	16 (51.6%)				
BMI (percentile)[b]	61.0 (36.5–80.0)	25.5 (1–67)	1.019 (0.997–1.042)	0.108		
System involved						
Pure hepatic, *n* (%)	14 (100%)	19 (61.3%)	1.632 (1.233–2.158)	0.009		0.958
Hepatic + Neuro, *n* (%)	0 (0%)	12 (38.7%)				
Type of hepatic involvement[a]						
Decompensated, *n* (%) (dCLD + ACLF)	14 (100%)	16 (51.7%)	1.938 (1.378–2.724)	0.001		0.950
Compensated cCLD, *n* (%)	0 (0%)	15 (48.4%)				
Calculated PELD score[b]	−10.4 (−12.2 to −6.1)	−7.8 (−9.6 to −4.0)	0.906 (0.788–1.042)	0.168		
Duration of combination therapy[b] (months)	36.0 (24–60)	33.0 (24–60)	0.990 (0.967–1.013)	0.384		
Time required for normalization of AST and ALT (months)[a]	12.0 (11.5–18.0)	11.5 (5.0–14.0)	1.221 (1.028–1.449)	0.023		0.113
Total duration of normal transaminases before stopping D-pen (months)	17.0 (10.7–48.0)	22.0 (17.0–43.0)	0.987 (0.964–1.011)	0.297		
Time required for normalization of serum albumin (months)[a]	5.5 (4.0–7.0)	8.0 (5.5–12.0)	0.880 (0.742–1.044)	0.144		
Time required for UCE to ≤500 mcg/day (months)[a]	12.0 (12.0–19.5)	12 (12–12)	1.003 (0.940–1.070)	0.920		
Dose of D-pen (mg/kg/day)[b]	11.9 (9.5–16.5)	8.9 (6.0–13.8)	1.144 (1.012–1.293)	0.031		0.242
AST (U/L)[b]	37.9 (34.5–50.5)	35.0 (29.0– 42.0)	1.047 (0.992–1.104)	0.097		
ALT (U/L)[b]	44.0 (42.0–54.0)	31.0 (25.0–43.8)	1.071 (1.020–1.125)	0.006	1.077 (1.014–1.145)	0.017
Total bilirubin (mg/dL)[b]	0.53 (0.41–0.91)	0.71 (0.50–1.0)	0.499 (0.091–2.722)	0.422		
Serum albumin (g/dL)[b]	4.34 (4.13–4.47)	4.29 (4.10–4.46)	1.602 (0.209– 12.283)	0.650		
INR[b]	1.17 (1.08–1.23)	1.18 (1.08–1.28)	1.621 (0.008–32.93)	0.859		
UCE (mcg/day)[b]	260.0 (125.5–447.7)	210.7 (61.0–319.0)	1.003 (0.999–1.006)	0.198		
NCC (mcg/dL)[b]	−0.37 (−1.8 to 4.96)	0.03 (−1.21 to 3.07)	1.018 (0.939–1.103)	0.664		
Transient elastography (kPa)[b]	11.5 (9.8–16.8)	11.4 (6.9–18.3)	0.996 (0.920–1.078)	0.925		
Hemoglobin (g/dL)[b]	12.1 (11.9–13.0)	12.5 (11.2–13.2)	0.841 (0.579–1.222)	0.363		

TABLE 2 (Continued)

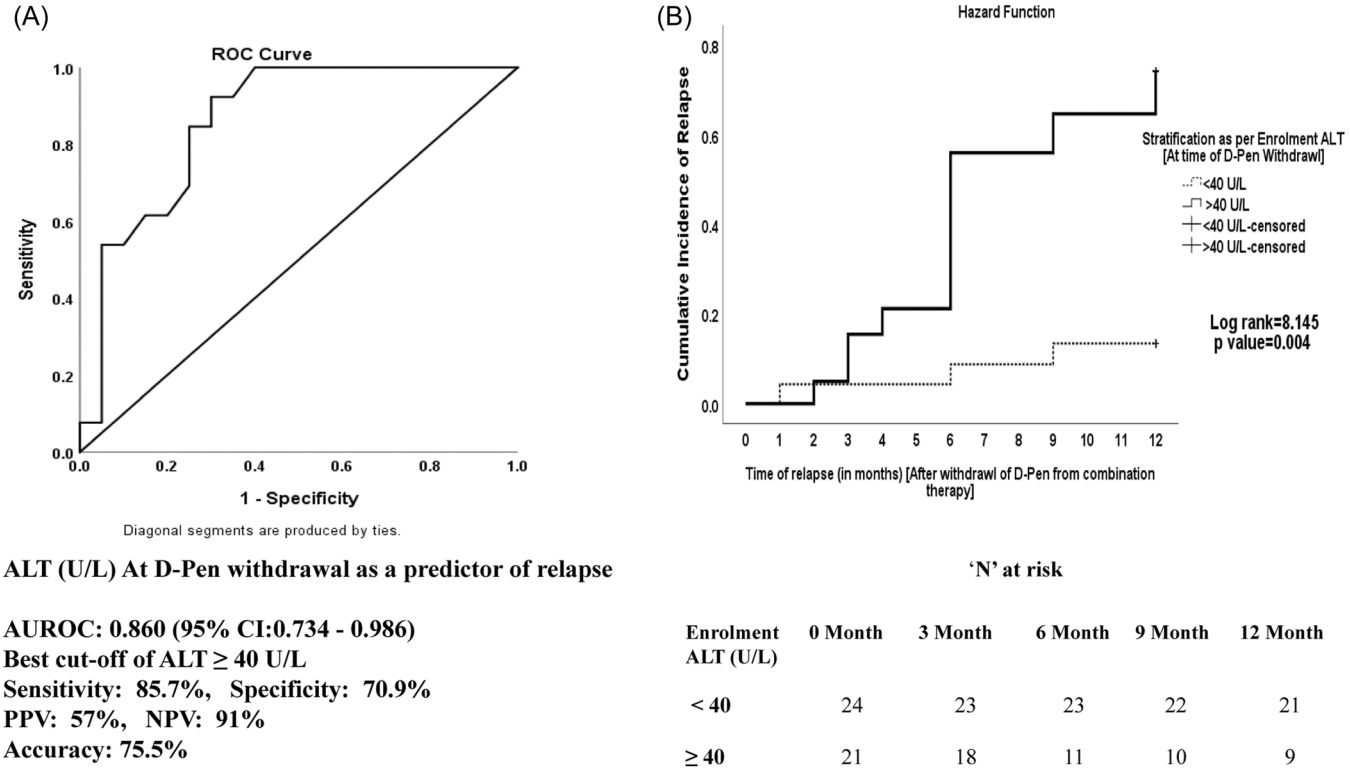
Variable	Relapsers ($n = 14$)	Nonrelapsers ($n = 31$)	Univariate analysis HR (95% CI)	*p*-Value	Multivariate analysis Adjusted HR (95% CI)	*p*-Value
Total leukocyte count (thousands/mm^3)[b]	5.6 (5.1–6.4)	5.1 (4.1–5.7)	1.334 (0.809–2.200)	0.258		
Total platelet count (Thousand/mm^3)[b]	157 (115–188)	150 (78–183)	1.000 (0.993–1.006)	0.878		

Note: Quantitative variables are expressed as median (IQR) and qualitative variables as percentage.

Abbreviations: ACLF, acute on chronic liver failure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; cCLD, compensated chronic liver disease; dCLD, decompensated chronic liver disease; D-pen, D-penicillamine; HR, hazard ratio; INR, international normalized ratio; IQR, interquartile range; kPa, kilo pascal; mcg, microgram; NCC, nonceruloplasmin bound; PELD, pediatric end-stage liver disease score; UCE, 24-h urine copper excretion.

[a]At index presentation (after starting combination therapy).

[b]At the time of D-pen withdrawal from combination therapy.



Enrolment ALT (U/L)	0 Month	3 Month	6 Month	9 Month	12 Month
< 40	24	23	23	22	21
≥ 40	21	18	11	10	9

FIGURE 2 (A) ROC curve of enrollment ALT as a predictor of relapse after withdrawal of D-pen from combination therapy in the maintenance phase of hepatic WD. (B) Kaplan–Meier hazard curve showing the difference in cumulative incidence of relapse in patients with ALT < 40 U/L and ≥40 U/L at the time of stoppage of D-pen. ALT, alanine aminotransferase; AUROC, area under receiver operating characteristic curve; D-pen, D-penicillamine; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic; WD, Wilson disease.

also an issue of anemia due to copper deficiency on long-term chelation therapy in patients whose UCE is persistently below 200 mcg/day.[20,30–32]

We stopped D-pen in 45 of the 94 patients screened and prospectively followed them for 12 months. The relapse rate was 31.8% in our study and majority (78%) occurred within 6 months of D-pen stoppage. The patient population and outcome of various earlier studies, where WD patients were shifted from either combination/chelator monotherapy to Zn monotherapy are summarized in SDC-S4. There is only one previous study by Shimizu et al. that prospectively evaluated the outcome of 37 patients (17 children), who had been shifted from chelator/combination therapy to Zn monotherapy. Follow-up of the cohort up to 48 weeks did not reveal any significant change in transaminases, though minor fluctuations in ALT up to 70–80 U/L were documented.[24] Ranucci et al. also demonstrated

feasibility of shifting D-pen responders to Zn monotherapy once the liver biochemical tests normalize, without any relapse. However, it is important to note that, they did not include any patient with dCLD as index presentation.[25] Gupta et al. had reported a cohort of 31 hepatic WD of age 5–24 years (10 had associated neurological symptoms, 54.8%: Child C grade) who were switched over to Zn after initial treatment with D-pen for 134 weeks (range: 2–320 weeks). During the follow-up period of 363 (35–728) weeks, three patients (10%) succumbed due to decompensation of liver disease; whereas rest remained stable on Zn alone.[26] There is a single pediatric study by Chang et al. where they shifted 65 patients from combination therapy to Zn monotherapy.[33] However, detailed follow-up of these patients has not been described in their results.

The inclusion criteria of the present study were designed drawing insights from an earlier study including our pediatric Wilson cohort, where 80% of compliant patients achieved "Criteria for adequate chelation (CAC)" within 1–2 years of combination therapy.[34] By choosing those "CAC," as inclusion criteria, it was ensured that all recruited patients had been adequately chelated before D-pen stoppage. Children with mildly elevated liver enzymes (40–60 U/L) were also included (only if they fulfilled all other criteria) to ascertain whether transaminase levels at D-pen stoppage have predictive value for relapse and if so, then to establish a cut-off for such prediction.

The incidence of relapse has also not been uniformly evaluated in the previous studies because of a lack of well-defined end-points/criteria for relapse. Ours is the first one to assess the biochemical and clinical parameters prospectively and objectively after D-pen cessation from combination therapy. Eighty-five percent of relapsers in the present study achieved their baseline AST/ALT within 3 months of reintroducing D-pen, which further affirms that relapses have actually occurred due to D-pen stoppage.

Moreover, none of the earlier studies have evaluated the predictors of relapse. In our study, baseline serum ALT at D-pen stoppage was the only independent predictor of relapse. An ALT of ≥40 U/L predicted risk of relapse with 85.7% sensitivity, 70.9% specificity. Only two out of 24 subjects (8.3%) with ALT <40 U/L had a relapse whereas more than half of children with ALT ≥40 U/L (12 out of 21) developed a relapse in our study. Thus, ALT had an excellent NPV (91%) that is, only 1 out of 11 children with ALT persistently <40 U/L has a relapse risk. It is essential to identify the cohort of patients who can be safely transitioned from chelator + Zn combination therapy to Zn monotherapy. Hence, it is prudent to mention here that, in our cohort, all relapses happened only in those patients who had their initial presentation as decompensated cirrhosis. Moreover, none of the patients with combined (hepatic + neurological) WD relapsed, despite 4/12 (33%) of them having ALT between 40 and 60 U/L. This is in concordance with Sinha et al., where no relapses occurred after D-pen discontinuation in predominantly neurological WD patients[27] and suggests that patients with combined (hepatic + neurological) WD could possibly be safely transitioned to Zn monotherapy in maintenance phase.

High mortality (10%) in the cohort of Gupta et al. who were initially dCLD, mandates extreme caution while discontinuing D-pen in these patients.[26] In our study, one patient who had initial presentation as dCLD, developed coagulopathy without elevation of transaminases on stopping D-pen, following which he was reintroduced to chelator. Early identification of relapsers by predefined regular biochemical follow-up and timely addition of D-pen could be the reason for maintained synthetic liver functions (serum albumin, INR) and absence of further decompensation in the relapsers in the present study.

Relapsers had a rise in NCC and UCE at relapse in contrast to nonrelapsers, in whom copper studies continued to remain normal throughout follow-up. This is suggestive of an increase in copper load following cessation of D-pen in relapsers; might be attributed to the lower decoppering potential of Zn. However, we found that increase in NCC and UCE was concurrent to elevation in AST/ALT and did not precede the rise in transaminases. Hence, increase in AST/ALT is earliest biochemical evidence of relapse.

Our study shows that if D-pen is withdrawn from combination therapy, 32% are likely to relapse. Hence it should be done in a very carefully selected cohort of patients, who would remain under stringent clinical and biochemical follow-up at least for 1–2 years after discontinuation of D-pen. Those who are likely to be noncompliant for close follow-ups should not be given trial of D-pen withdrawal. Additionally, we felt that 6 months duration of biochemical remission is short, especially for children with decompensated liver disease at diagnosis. These children might require a longer duration of biochemical remission before considering for cessation of D-pen. Moreover, withdrawal of D-pen by gradually tapering the dose (especially for those receiving higher dosage) with close monitoring of biochemical response might be a more judicious approach rather than stopping it at once.

The strength of our study is the strict inclusion criteria and well-defined endpoints for relapse. This is the first prospective study in pediatric age group to evaluate the incidence and predictors of relapse following withdrawal of D-pen from combination therapy in maintenance phase treatment of WD. Limitations are a smaller sample size as it is a single-center study and shorter duration of follow-up. Since there is a chance that a few nonrelapsers might relapse after 1 year, we have kept them in close follow-up.

To conclude, in a very selective cohort of pediatric hepatic WD, it might be possible to withdraw D-pen and hence shift to Zn monotherapy during maintenance

phase; those with associated neurological WD and those who never decompensated with ALT persistently below 40 U/L after initiation of treatment may be the ideal candidates for withdrawal of D-pen in a controlled setting.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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