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Original Article

# Hereditary tyrosinemia type 1 in Turkey: Twenty year single-center experience

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**Abstract** ***Background:*** Hereditary tyrosinemia type 1(HT1) is a chronic disorder leading to severe hepatic, renal and peripheral nerve damage if left untreated. Despite nitisinone treatment HT1 still carries the risks of hepatocellular carcinoma (HCC) and neuropsychological outcome.

***Methods:*** A retrospective single center study was carried out based on the phenotype, therapy and outcome in 38 Turkish patients with HT1 diagnosed during the last 20 years.

***Results:*** None of the patients was diagnosed on newborn screening. The patients were grouped according to acute, subacute and chronic forms of the disorder. The main clinical manifestations were hepatosplenomegaly, liver and renal tubular dysfunction. Thirty-six patients were treated with nitisinone. The mean duration of nitisinone treatment was 64 months and the mean dosage was 1.2 mg/kg/day. Dietary compliance problems were frequent. Eleven patients had cognitive evaluation (mean total IQ, 84 points). Six patients had living donor liver transplantation despite nitisinone treatment: three due to suspected HCC, two for non-compliance to diet, and one for both, at a median age of 90 months.

***Conclusion:*** Nitisinone treatment is effective and improves both short- and long-term prognosis of HT1. Early diagnosis on newborn screening is needed because delay in treatment increases the risk of the persistence of hepatic disease and HCC. Interruption of the drug can lead to re-occurrence of hepatocellular damage and neurological crisis. Increased α-fetoprotein and new hypoechoic nodule formation are the warning signs for HCC.

**Key words** hepatocellular carcinoma, liver transplantation, NTBC, Turkey, tyrosinemia type 1.

Hereditary tyrosinemia type 1 (HT1, OMIM 276700) is a rare autosomal recessive disorder caused by deficiency of the last enzyme in the tyrosine (Tyr) catabolic pathway, fumarylacetoacetate hydrolase (FAH).[1,2] Blockage leads to accumulation of the toxic metabolites fumarylacetoacetate (FAA), maleylacetoacetate (MAA), succinylacetoacetate (SAA), and succinylacetone (SA), which are responsible for progressive hepatic, renal and neurological findings.[3,4] The human FAH complementary DNA has been cloned and mapped to human chromosome 15q.[5]

The clinical manifestations of HT1 are heterogeneous, even within the members of the same family. The disorder is classified based on age at symptom onset: acute, <6 months; subacute, 6 months–1 year; and chronic, >1 year of age.[1,6] Acute/chronic liver disease and renal tubular dysfunction with Fanconi syndrome leading to secondary hypophosphatemic rickets are the main clinical findings.[7,8] Hepatic lesions are responsible for the long-term complications: micro/macronodular cirrhosis and hepatocellular carcinoma (HCC).[9] Polyneuropathy and abdominal pain similar to acute porphyria,[4] cardiomyopathy and hyperinsulinism are also described in untreated patients.[1,2,9–11]

The natural history of the disease usually results in death.[12,13] Liver transplantation (LT) was the first definitive therapy introduced.[1] Low Tyr-phenylalanine (Phe) diet was not effective in the acute form and had limited success in the chronic form, nor did it prevent HCC. 2-(2-Nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC; nitisinone) is a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase, leading to the suppression of MAA, FAA and SA accumulation and the accumulation of Tyr.[14,15] Nitisinone had significantly improved survival in HT1, with a clinical response of 90% and a decrease in the risk of early development of HCC in those who began treatment at an early age.[16–22]

In the present study we investigated the clinical and biochemical features of 38 Turkish HT1 patients. The aim was to increase the knowledge on clinical outcome in these patients, the consequences of late and interrupted treatment and improve the

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understanding of current diagnosis and clinical management practices for HT1 in countries where expanded newborn screening is not part of the newborn screening program.

## Methods

### *Patients*

We reviewed all patients with HT1 diagnosed in the Nutrition and Metabolic Diseases Unit, Istanbul University Cerrahpasa Children's Hospital in the 20 year period from December 1993 to January 2014. A detailed history was taken from all subjects. Clinical and laboratory evaluation including full blood count, urine analysis, liver and renal function tests, coagulation profile, serum calcium, phosphate, alkaline phosphatase (ALP), plasma quantitative amino acid, urine organic acid and delta-aminolevulinate (δ-ALA) analysis, abdominal ultrasound and cardiac echocardiography were performed at the time of presentation. Diagnosis of HT1 was made on detection of elevated SA in urine/blood samples. Abdominal computed tomography (CT) and/or magnetic resonance imaging (MRI) were performed on the basis of suspected HCC.

As soon as the diagnosis had been confirmed all of the patients were started on a Tyr-Phe restricted diet under the supervision of a clinical dietician to maintain plasma Tyr <400 μmol/L as recommended.[1] Two patients were treated only with diet. Thirty-six patients received nitisinone therapy divided in one or two doses along with the diet.[2,18,23]

All patients were followed up with both clinical and laboratory investigations. Liver and renal parenchymal changes were followed on ultrasound and on abdominal CT or MRI if HCC was suspected. Ocular side-effects were followed up on ophthalmological examination. Neurocognitive development was followed on Denver developmental screening test. Intelligence quotient (IQ) was assessed in those with parental permission.

Non-parametric Mann–Whitney *U*-test was used for abnormally distributed data. $P < 0.05$ was considered statistically significant. Results are expressed as mean, median and range.

## Results

### *Clinical and biochemical data*

Thirty-eight HT1 patients aged between 15 days and 100 months at the time of diagnosis were studied. Fourteen were female, 24 were male. None of the cases of HT1 was detected on newborn screening test. Mean age at onset of clinical symptoms was 9 months (range, 0–54 months). Very early onset of symptoms (≤2 months) was noted in 12 cases (31%), early onset in 16 cases (2–6 months), and late onset (>24 months) in seven cases. Mean age at diagnosis was 19 months (range, 0.5–100 months). Thirteen patients were diagnosed with acute, 12 with subacute and 13 with chronic HT1.

Medical history, chief complaints and clinical findings at presentation are summarized in Table 1, Figures 1,2. The median age at initial complaints was 2 months (range, 0–4.5 months) in acute, 3.6 months (range, 1–7.5 months) in subacute and 20 months (range, 2–54 months) in chronic HT1, respectively, while the median interval between the first complaints and diagnosis was 1.3 months (range, 0–3.1 months) in acute, 6.2 months (range, 0.5–11 months) in subacute, and 17 months (range, 1–67 months) in chronic HT1, respectively. The interval was statistically significantly different between the groups ($P = 0.0002$ between acute and subacute HT1; $P < 0.0001$, acute and chronic; and $P = 0.0001$, subacute and chronic). The delay was most prominent in the chronic form.

**Table 1** Medical history

| Medical history | *n* (%) |
|---|---|
| Consanguinity | 24 (63.1) |
| Family history | |
| Sibling with HT1 | 6 (15.8) |
| History of sibling death (unexplained) | 5 (13.2) |
| Cirrhosis in sibling | 3 (8) |
| Cousin with tyrosinemia type 1 | 3 (8) |
| Cirrhosis in relative | 1 (2.6) |
| Hepatocellular carcinoma in relative | 1 (2.6) |
| Birth history | |
| Prematurity (weeks of gestation) | 6 (16) |
| 36 | 3 (8) |
| 35 | 1 (2.6) |
| 33 | 1 (2.6) |
| 28 | 1 (2.6) |
| Small for gestational age | 1 (2.6) |
| LGA due to gestational diabetes | 3 (8) |
| Neonatal hypoglycemia | 2 (5.3) |

HT1, hereditary tyrosinemia type 1; LGA, large for gestational age.

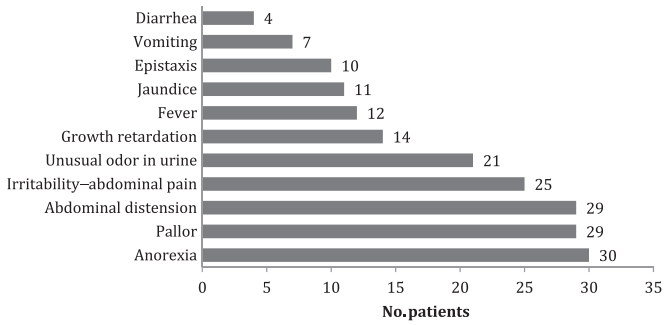


**Fig. 1** Chief clinical symptoms at presentation.

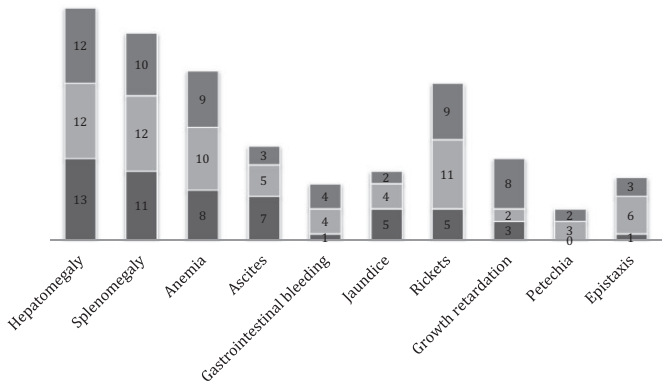


**Fig. 2** Clinical findings at presentation. ■, acute hereditary tyrosinemia type 1 (HT1); ■ subacute HT1; ■, chronic HT1.

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On birth history, prematurity was noted in six patients. Four were moderate/late preterm (32–<37 weeks) and one was very preterm (28 weeks). None of them had any other detected risk factor for premature labor.

On initial anthropometric evaluation five patients were <–2 standard deviation score (SDS) for height and six were <–2SDS for weight.

On initial laboratory evaluation increase of aspartate aminotransferase (AST; >40 IU/L) was noted in 29 patients and of alanine aminotransferase (ALT; >40 IU/L) in 10 patients. Activated partial thromboplastin time was >40 s in 32/38, and international normalized ratio was >1.3 in 30/38. All patients had elevated α-fetoprotein (AFP) for age, of highly variable degree. Erythrocyte porphobilinogen synthase (ePBGS) was markedly decreased. Plasma SA, urine SA, Tyr, Phe, methionine and urinary δ-aminolevulinic acid were increased. Tubulopathy was detected in 36/38 (94.7%): generalized aminoaciduria in 36, glycosuria in 21, metabolic acidosis in eight patients. Clinical rickets was detected in 25 patients (66%), of whom four had complaints. Hypophosphatemia was detected in 16, hypocalcemia in nine and increased ALP in 31 patients. Urinary phosphate results were available for 13 children at presentation; 12/13 had abnormal tubular phosphate reabsorption. Eleven patients were found to be hypoglycemic at the time of presentation: two were hyperinsulinemic and needed temporary i.v. dextrose infusion (3–4 mg/kg/min), others responded to oral feeding. Biochemical data and differences in laboratory findings between the groups are summarized in Tables 2,3.

On renal ultrasonography, nephromegaly was noted in 21 (55%), increased echogenicity in 22 (58%) and nephrocalcinosis in four patients (10.5%). Hepatic ultrasonography showed hepatomegaly in 36 (94.6%) and splenomegaly in 32 patients (84%). Hepatic architecture was heterogeneous with granular appearance in 33/38 patients (87%). A total of 26/38 (68.4%) had hepatic surface nodularity (15 of these patients were <1 year of age, and eight <6 months of age), 19 (50%) had multiple hypoechoic nodules, 20 (53%) had multiple hyperechoic nodules and five (5%) had macronodular appearance detected on either ultrasonography or MRI even at the time of presentation. Liver biopsy was performed in 13 patients at the time of presentation and all had active cirrhosis.

Echocardiography was available in 15 patients. Eleven were normal, one had mild interventricular septal hypertrophy with mild mitral insufficiency, one had small muscular ventricular septal defect, one had ostium secundum atrial septal defect and one had patent foramen ovale with mild pulmonary stenosis.

### *Response to treatment*

Table 4 lists the results of long-term follow up. Both of the patients treated with low Tyr/Phe diet only, died due to severe hepatic dysfunction and massive bleeding, at 11 and 23 months of age, respectively. Thirty-six patients were treated with nitisinone

**Table 2** Laboratory data at presentation

| Parameter | Normal | *n* | Mean | SD | Range |
|---|---|---|---|---|---|
| Plasma | | | | | |
| AFP (μg/L) | <13 | 38 | 96 800 | 140 566 | 35–624 000 |
| ALT (IU/L) | 0–40 | 38 | 34.4 | 21.8 | 11–102 |
| AST (IU/L) | 0–40 | 38 | 71.4 | 32.9 | 25–186 |
| GGT (IU/L) | 3–25 | 38 | 119 | 73.9 | 190–269 |
| Ca (mg/dL) | 8.4–10.8 | 38 | 9.1 | 0.91 | 7.51–11.1 |
| P (mg/dL) | 2.7–5.5 | 38 | 3.07 | 1.36 | 1.20–6.10 |
| ALP (IU/L) | 60–525 | 38 | 1 490 | 1 105 | 170–4 430 |
| Total protein (g/dL) | 5.6–8 | 38 | 5.7 | 1.14 | 3.9–8.3 |
| Albumin (g/dL) | 3.2–5.4 | 38 | 3.37 | 0.72 | 2.1–4.7 |
| Plasma | | | | | |
| ePBGS (nkat/g Hb) | 0.58–1.25 | 17 | 0.071 | 0.10 | 0.0–0.32 |
| SAp (μmol/L) | <0.1 | 19 | 34.5 | 33.8 | 0.73–136 |
| TYR (μmol/L) | 50–130 | 38 | 409 | 291.7 | 23–1 095 |
| PA (μmol/L) | 40–120 | 38 | 133 | 103.4 | 15–415 |
| Met (μmol/L) | 20–50 | 38 | 371.8 | 398.3 | 13.3–1 590 |
| Urine | | | | | |
| SA (mmol/mol creatinin) | <1 | 19 | 503 | 560 | 1.4–1 800 |
| DALA (mmol/mol creatinin) | 0–3 | 16 | 123.6 | 111.7 | 11–350 |
| SA (qualitative) | None | 19 | | Increased | |
| Coagulation | | | | | |
| PT (s) | 10.4–14 | 38 | 30.3 | 15.07 | 13.3–70 |
| PT activity (%) | 70–130 | 38 | 37.14 | 21.8 | 11.3–90 |
| INR | 0.85–1.15 | 38 | 2.67 | 1.35 | 1.05–6.15 |
| aPTT (s) | 26–40.8 | 38 | 63.4 | 25.4 | 14.8–127 |

AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine transaminase; aPTT, activated partial thromboplastin time; AST, Aspartate transaminase; Ca, calcium; DALA, δ-aminolevulinic acid; ePBGS, erythrocyte porphobilinogen synthase; GGT, γ-glutamine transaminase; INR, international normalized ratio; Met, methionine; P, phosphorus; PA, phenylalanine; PT, prothrombin time; SA, succinylacetone; SAp, plasma succinylacetone; TYR, tyrosine.

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**Table 3** Laboratory findings vs type of HT1

| | Acute ($n$ = 13) | Subacute ($n$ = 12) | Chronic ($n$ = 13) | Significance |
|---|---|---|---|---|
| ALT (IU/L) | **25.5 ± 13.6 (11–54)[a]** | **45.1 ± 25.3 (16–102)** | **33.4 ± 22.1 (14–79)** | **[a]*P* = 0.03** |
| AST (IU/L) | 62 ± 16.9 (35–85) | 84.8 ± 42.12 (25–186) | 68.1 ± 34 (29–125) | NS |
| GGT (mg/dL) | 123 ± 72.8 (27–255) | 117 ± 76 (31–256) | 116.4 ± 81 (19–269) | NS |
| Total bilirubin (mg/dL) | **1.9 ± 1.04 (1–3.97)** | **2.62 ± 1.42 (1.24–6.1)[c]** | **1.42 ± 1.28 (0.41–4.59)** | **[c]*P* = 0.016** |
| Direct bilirubin (mg/dL) | **0.98 ± 0.57 (0.27–2.20)** | **1.31 ± 0.81 (0.39–3.30)[c]** | **0.61 ± 0.62 (0.06–2.15)** | **[c]*P* = 0.01** |
| Prothrombin time (s) | **37.4 ± 18.9 (14.8–70)[b]** | **35.2 ± 10.6 (24.3–54.4)[c]** | **18.3 ± 3.81 (13.3–27.7)** | **[b]*P* = 0.008 [c]*P* < 0.0001** |
| Prothrombin activity (%) | **31.4 ± 21.5 (12–80)[b]** | **22.87 ± 7.92 (11.34–36.1)[c]** | **56.16 ± 17.3 (30.5–90)** | **[b]*P* = 0.006 [c]*P* < 0.0001** |
| APTT (s) | **74.4 ± 27.7 (37.8–127)[b]** | **71.5 ± 22.4 (45–119.8)[c]** | **46.16 ± 10.25 (29.9–71.8)** | **[b]*P* = 0.002 [c]*P* < 0.0001** |
| INR | **3.23 ± 1.62 (1.13–6.15)[b]** | **3.28 ± 0.922 (1.91–4.75)[c]** | **1.5 ± 0.32 (1.05–2.13)** | **[b]*P* = 0.006 [c]*P* < 0.0001** |
| Urea (mg/dL) | **13.08 ± 6.97 (3–28)** | **11.71 ± 4.57 (6–20)[c]** | **25.92 ± 22.64 (4–90)** | **[c]*P* = 0.046** |
| Creatinine (mg/dL) | 0.34 ± 0.1 (0.1–0.5) | 0.36 ± 0.12 (0.2–0.67) | 0.38 ± 0.14 (0.2–0.64) | NS |
| Calcium (mg/dL) | **9.65 ± 0.80 (8.4–11.1)[a]** | **8.79 ± 0.92 (7.6–10.7)** | **8.92 ± 0.83 (7.51–10)** | **[a]*P* = 0.032** |
| Phosphorus(mg/dL) | 35 ± 1.58 (1.37–6.10) | 2.61 ± 1.16 (1.2–4.9) | 3.05 ± 1.25 (1.2–4.9) | NS |
| ALP (IU/L) | **1 415 ± 1 208 (233–4 108)[a]** | **2 216 ± 1 101 (1 007–4 430)[c]** | **825 ± 398 (170–1 460)** | **[a]*P* = 0.039 [c]*P* < 0.0001** |
| Hemoglobin (g/dL) | 10.3 ± 2.87 (7.6–19) | 9.04 ± 1.5 (7.1–11.2) | 9.8 ± 2.47 (6.4–13.9) | NS |
| Leukocyte ($mm^3$) | **10 946 ± 4 668 (4 100–22 900)[b]** | **8 820 ± 4 215 (3 250–18 900)** | **7 610 ± 2 998 (4 250–12 900)** | **[b]*P* = 0.035** |
| Thrombocyte ($mm^3$) | 119 000 ± 36 424 (79 000–181 000) | 115 000 ± 42 464 (53 000–21 100) | 152 000 ± 139 389 (33 000–577 000) | NS |
| α-Fetoprotein (μL/L) | **144 000 ± 171 958 (370–624 000)[b]** | **141 000 ± 141 588 (1 530–450 000)[c]** | **9 160 ± 17 831 (35–65 500)** | **[b]*P* < 0.0001 [c]*P* < 0.0001** |
| ePBGS (nkat/g Hb) | 0.09 ± 0.13 (0.00–0.32) | 0.08 ± 0.1 (0.01–0.28) | 0.036 ± 0.02 (0.02–0.06) | NS |
| SAp (μmol/L)(<0.1) | 51.8 ± 43.7 (3.60–136) | 29.8 ± 24.15 (0.73–77) | 19.8 ± 23.75 (3.9–54.7) | NS |
| Tyrosine (μmol/L) | 386 ± 230 (112–830) | 447 ± 357.3 (23–1 095) | 394 ± 272 (137–836) | NS |
| Phenylalanine (μmol/L) | 171 ± 127.8 (15–415) | 1 280 ± 101.4 (24–346) | 98.6 ± 64.2 (51–255) | NS |
| Methionine (μmol/L) | **533 ± 385.4 (48–1 108)[b]** | **477.7 ± 447.4 (70–1 590)[c]** | **63.1 ± 52.6 (13.3–158)** | **[b]*P* = 0.001 [c]*P* = 0.001** |
| Urine DALA (mmol/mol creatinin) | **250 ± 116.2 (104–350)[a,b]** | **90.4 ± 82.8 (16–243)** | **54.3 ± 40.4 (11–91)** | **[a]*P* = 0.039 [b]*P* = 0.034** |
| SAu (mmol/mol creatinin) | 709 ± 476.2 (5.9–1 400) | 554 ± 663 (1.4–1 800) | 80 ± 46.3 (12–116) | NS |

[a]Acute vs subacute; [b]acute vs chronic; [c]subacute vs chronic. ALP, alkaline phosphatase; ALT, alanine transaminase; APTT, activated partial thromboplastin time; AST, aspartate transaminase; DALA, δ-aminolevulinic acid; ePBGS, erythrocyte porphobilinogen synthase; GGT, γ-glutamine transaminase; HT1, hereditary tyrosinemia type 1; INR, international normalized ratio; NS, not significant; SAp, plasma succinylacetone; SAu, urine succinylacetone. Statistically significant values as expressed in bold.

and diet. The mean interval between diagnosis and nitisinone treatment was 20 days (range, 0–120 days). Initiation of nitisinone treatment was done at <6 months of age in 14 patients (39%), 7–12 months in nine patients (25%), 13–24 months in two patients (5.5%) and after 24 months in 11 patients (30.5%). Long-term follow up was carried out for a period of 1.5–239 months (mean, 67.4 months): in eight for <6 months (22%), in two for 7–12 months (5.5%), in seven for 13–48 months (19%), and in 19 for 49–239 months (53%).

The mean dosage of nitisinone was 1.2 mg/kg/day (range 0.6–2 mg/kg/day) with an average plasma level of 41 μmol/L. The dose was adjusted according to the plasma nitisinone level

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**Table 4** Clinical outcome of HT1

| Patient no. | HT1 type | Treatment | Age at onset of symptoms (months) | Age at diagnosis (months) | Duration between diagnosis and treatment (months) | Length of follow up (months) | Result |
|---|---|---|---|---|---|---|---|
| A1 | Acute | NTBC + Low Tyr-Phe diet | 1 | 3 | 5 | 5.65 | **Died** (due to septic shock) |
| A2 | Acute | NTBC + Low Tyr-Phe diet | 2.4 | 3.4 | 0 | 113 | Alive |
| A3 | Acute | NTBC + Low Tyr-Phe diet | 3.05 | 3.12 | 0 | 67 | Alive |
| A4 | Acute | NTBC + Low Tyr-Phe diet | 1 | 2.8 | 18 | 101.5 | Alive |
| A5 | Acute | NTBC + Low Tyr-Phe diet | 1.5 | 2.3 | 0 | 75 | Alive |
| A6 | Acute | NTBC + Low Tyr-Phe diet | 2 | 4.27 | 0 | 79 | Alive |
| A7 | Acute | NTBC + Low Tyr-Phe diet | 2 | 3 | 0 | 48 | Alive |
| A8 | Acute | NTBC + Low Tyr-Phe diet | 2 | 5 | 15 | 5 | Alive (partial response to treatment, waiting for LT) |
| A9 | Acute | NTBC + Low Tyr-Phe diet | 0 | 0.43 | 0 | 4 | Alive |
| A10 | Acute | NTBC + Low Tyr-Phe diet | 3 | 3 | 0 | 57 | Alive |
| A11 | Acute | NTBC + Low Tyr-Phe diet | 4.1 | 4.14 | 0 | 22 | Alive |
| A12 | Acute | NTBC + Low Tyr-Phe diet | 4.5 | 5.85 | 8 | 128 | Alive |
| A13 | Acute | NTBC + Low Tyr-Phe diet | 2 | 5.09 | 7 | 156 | Alive |
| S1 | Subacute | NTBC + Low Tyr-Phe diet | 3.5 | 8.2 | 30 | 2.7 | **Died** (non-responder; hepatic insufficiency with increasing jaundice and intractable coagulopathy despite 5 months of therapy) |
| S2 | Subacute | NTBC + Low Tyr-Phe diet | 7.5 | 8 | 120 | 12 | **Died** (HCC; died at 8th month of treatment at 22 months of age) |
| S3 | Subacute | Low Tyr-Phe diet | 3.5 | 12.6 | 0 | 2 | **Died** (massive bleeding due to hepatic insufficiency) |
| S4 | Subacute | NTBC + Low Tyr-Phe diet | 3.75 | 6.47 | 6 | 25 | **Died** (due to porphyria-like attack, during interruption of treatment for 8 months) |
| S5 | Subacute | NTBC + Low Tyr-Phe diet | 1 | 8.18 | 120 | 102 | Alive |
| S6 | Subacute | NTBC + Low Tyr-Phe diet | 6 | 6.6 | 5 | 111 | Alive |
| S7 | Subacute | NTBC + Low Tyr-Phe diet | 5 | 7.06 | 18 | 62 | Alive |
| S8 | Subacute | NTBC + Low Tyr-Phe diet | 4 | 12 | 8 | 112 | Alive |
| S9 | Subacute | NTBC + Low Tyr-Phe diet | 6 | 13 | 26 | 188 | Alive (LDLT; HCC detected at transplantation) |
| S10 | Subacute | NTBC + Low Tyr-Phe diet | 2.5 | 8 | 12 | 228 | Alive |
| S11 | Subacute | NTBC + Low Tyr-Phe diet | 1 | 9 | 0 | 44 | Alive (LDLT; after 46 months of treatment, due to suspected HCC. HCC was detected at transplantation. |
| S12 | Subacute | NTBC + Low Tyr-Phe diet | 1 | 12 | 0 | 2 | Alive (On the list for LT due to partial response) |
| C1 | Chronic | Low Tyr-Phe diet | 6 | 23 | 0 | 2 | **Died** (Hepatic insufficiency and bleeding esophageal varices) |
| C2 | Chronic | NTBC + Low Tyr-Phe diet | 8 | 19 | 0 | 30 | Alive |
| C3 | Chronic | NTBC + Low Tyr-Phe diet | 4 | 27 | 30 | 62 | Alive (LDLT, after 63 months of treatment, due to non-compliance with treatment and suspected HCC; HCC detected at transplantation) |
| C4 | Chronic | NTBC + Low Tyr-Phe diet | 24 | 33.4 | 30 | 12 | **Died** (permanent cessation of NTBC treatment due to parents' decision led to death due to hepatic insufficiency and esophageal variceal bleeding at 4 months of treatment) |
| C5 | Chronic | NTBC + Low Tyr-Phe diet | 12 | 26.5 | 12 | 76 | Alive |
| C6 | Chronic | NTBC + Low Tyr-Phe diet | 36 | 60 | 30 | 55 | **Died** (permanent cessation of NTBC treatment due to parents' decision led to death due to HCC at 27 months of treatment) |
| C7 | Chronic | NTBC + Low Tyr-Phe diet | 2 | 50 | 57 | 115 | Alive (LDLT due to suspected HCC, after 62 months of treatment, dysplasia detected at transplantation) |
| C8 | Chronic | NTBC + Low Tyr-Phe diet | 40 | 48.7 | 0 | 2.2 | **Died** (partial response, death due to portal hypertension and massive variceal bleeding at 10th month of treatment) |
| C9 | Chronic | NTBC + Low Tyr-Phe diet | 5 | 72 | 15 | 227 | Alive |
| C10 | Chronic | NTBC + Low Tyr-Phe diet | 54 | 100 | 30 | 4 | **Died** (metastatic HCC; died during chemotherapy at 5th month of treatment) |
| C11 | Chronic | NTBC + Low Tyr-Phe diet | 24 | 30 | 15 | 41 | Alive (LDLT, after 34 months of treatment due to non-compliance with dietary treatment) |
| C12 | Chronic | NTBC + Low Tyr-Phe diet | 38 | 39 | 90 | 50 | Alive (LDLT, after 47 months of treatment non-compliance with dietary treatment) |
| C13 | Chronic | NTBC + Low Tyr-Phe diet | 20 | 43 | 1 | 2 | Alive (waiting for LT due to suspected HCC) |

HCC, hepatocellular carcinoma; HT1, hereditary tyrosinemia type 1; LDLT, living donor liver transplantation; LT, liver transplantation; NTBC, nitisinone; Tyr, tyrosine; Phe, phenylalanine. The patients died during the follow-up are expressed in bold.

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that was considered adequate (between 30 and 60 μmol/L). No urinary SA excretion was detected under nitisinone treatment, but interruption of the treatment led to re-excretion. Treatment adherence to nitisinone was very good in all except three chronic patients. Compliance with the dietary treatment was good in eight patients (Tyr <400 μmol/L), moderate in 14 (Tyr 400–600 μmol/L) and bad in 14 patients (Tyr >600 μmol/L).

Good metabolic control was achieved in 31 patients with normalization of the hypoprothrombinemia and decrease in AFP. One acute, one subacute and two chronic HT1 patients had only partial response to nitisinone treatment after 2, 3, 6 and 7 months of treatment, respectively. One of the chronic HT1 patients died due to variceal bleeding as a result of portal hypertension, and another two patients were under evaluation for living donor (LD) LT at the time of writing. One subacute HT1 patient did not respond to therapy and died due to hepatic insufficiency.

Among 27 patients who had been followed for >6 months, AFP normalized (<13 μg/L) in 4/27 (15%) within the first year of therapy, in 13/27 (48%) at 12–24 months, and in 7/28 (25%) at 25–48 months of therapy.

Two subacute and one chronic HT1 patients had persistent high AFP with normal liver function tests despite nitisinone treatment. All were diagnosed with HCC. One patient died during follow up, one had just undergone transplantation and one was being prepared for LT at the time of writing. Secondary increase in AFP after normalization was also detected in four patients (one acute, two subacute, one chronic). Only one responded to increase of nitisinone level, but the other three underwent transplantation: two had HCC and one had hepatic dysplasia detected at LT.

None of the 36 patients with tubulopathy had glomerular involvement, nor developed renal insufficiency. Two patients required temporary supplementation of bicarbonates. Rickets was cured in all 26 patients.

Porphyria-like crisis was not detected under regular nitisinone treatment. Interrupted nitisinone treatment for approximately 8 months resulted in death due to severe polyneuropathy with phrenic paresis and respiratory insufficiency in one subacute HT1 patient, and severe abdominal pain due to porphyria-like attack for 2 and 3 months of interruption, respectively, in two subacute HT1 patients.

The only patient with left ventricular septal hypertrophy responded well to nitisinone therapy with disappearance of the hypertrophy. Cardiomyopathy was not reported during the treatment.

Height and weight development normalized in 29 patients, but two patients remained at <2 SDS for height and five remained at <2 SDS for weight.

Overall, six patients underwent LDLT. The median age at transplantation was 90 months (range, 55–172 months) and the median age of treatment at the time of transplantation was 63 months (range, 33–159 months). Suspected HCC with normal liver function tests was the reason for LDLT in two subacute and one chronic HT1 patient. Serious difficulty in adherence to dietary treatment was the reason LDLT for two chronic HT1 patients; both maintained adequate nitisinone level (54.7 and 79.1 μmol/L, respectively) but had high plasma Tyr concentration (430 and 693 μmol/L, respectively). Non-compliance with both nitisinone and dietary treatment along with HCC suspicion was the reason for LDLT in one chronic HT1 patient. He had neither maintained adequate nitisinone nor Tyr (4.7 μmol/L and 567 μmol/L, respectively).

Two siblings, patients A11 and S7, had been followed up at another pediatric metabolic diseases unit after 22 months and 62 months of treatment, respectively.

No adverse effect required interruption of nitisinone treatment. Two patients complained of foreign body sensation in the eyes at plasma Tyr concentration 865 and 1290 μmol/L with subepithelial corneal opacities. Eye symptoms resolved with strict adherence to diet and decrease of plasma Tyr <400 μmol/L. No cutaneous lesions were detected. Two patients had transient leukopenia and two had transient thrombopenia without clinical consequences.

Eleven patients had cognitive evaluation: two undertook the age-appropriate Wechsler Scale IQ test (Wechsler Intelligence Scale for Children for age 7–17 years), eight were assessed with the Stanford-Binet Intelligence Scale, and one was assessed with the Cattell Culture Fair Intelligence Test. The mean total IQ was 84 (range 55–115). Low IQ score was associated with special education attendance. Stanford-Binet IQ test was repeated at a 3 year interval in only one patient, with a decline from 91 to 88. Nine patients were evaluated with the Denver II test. The mean total score was 86 (range, 60–100).

## Discussion

Hereditary tyrosinemia type 1 has a birth incidence of approximately 1 in 100 000 in most countries but is more common in Quebec, Canada due to the founder effect.[19] The exact incidence of HT1 in Turkey is unknown. Partly due to the high rate of consanguineous marriage (up to 20–25%), Turkey has a high estimated prevalence of inborn errors of metabolism.[24,25] The rate of consanguinity in the present cohort was high (63%) although lower than in Egypt, another country with a high rate of consanguineous marriage.[26] HT1 is not part of the newborn screening program, making it nearly impossible to diagnose a patient in the asymptomatic period in Turkey. None of the present patients was diagnosed in the newborn period, except one who was a sibling of another HT1 patient.

Due to severe clinical findings, the time between onset of symptoms and diagnosis was shortest in the acute HT1 group. Anorexia, pallor, abdominal distension and irritability with abdominal pain were the most common complaints. Most of the infants were diagnosed with infantile colic at the onset of symptoms. The insidious course of the disease resulted in delay in diagnosis in the chronic HT1 patients, as seen in previous reports.[1,12,13,27] The most striking difference was seen in a chronic HT1 patient, in whom symptoms started at 5 months of age but who was diagnosed at 6 years of age.

In all patients, the first manifestations of HT1 ranged from asymptomatic hepatomegaly to severe hepatic insufficiency, cirrhosis and HCC. Anemia and rickets were the other common clinical findings. Mild increase in ALT and AST with disruption

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of liver synthesis functions and elevated ALP and AFP were the most striking laboratory findings. The laboratory findings were more severe in acute HT1. Plasma Tyr was not always elevated, consistent with previous reports recommending using SA instead of Tyr in newborn screening of HT1.[20–22] Plasma and urine SA were increased in both mild and severe cases; 24 h urine collection was needed in one subacute HT1 patient. Although SA was lower in chronic cases, no significant difference was noted between groups.

The Tyr/Phe restricted diet alone was not effective and both patients died. In 36 patients the initial nitisinone dose ranged from 0.6 to 2 mg/kg/day in two divided doses, as described.[28,29] On follow up, the mean dose (1.2 mg/kg/day) was slightly higher than the standard recommendation of 1 mg/kg per day[27,28] and higher than the suggested dose in the El-Karaksy *et al*. and Couce *et al*. studies.[26,29,30] The mean nitisinone blood level was 41 μmol/L, within the recommended level, and was sufficient to eliminate SA excretion.[30–33]

Disruption of the hepatic architecture was a common finding along with nodularity even at the time of presentation, especially in late diagnosed patients. Nitisinone treatment was not effective in complete reversion of the hepatic lesions and normalization of the architecture, except in two patients. De novo hyperechoic and hypoechoic nodule formation were also detected despite nitisinone treatment without increase in AFP, although hypoechoic de novo nodule formation should always be an alert for HCC.

In the present cohort, complete unresponsiveness was noted in one subacute HT1 (S1) patient diagnosed at 8 months of age: although he was treated with nitisinone (2 mg/kg/day), he died due to progressive hepatic insufficiency in the second month of treatment. Two patients (A1, S12) had partial response to nitisinone treatment with partial recovery in the coagulation profile, and were under evaluation for LT after 6 months and 2 months of treatment, respectively. All of the non/partial-responder patients had severe hepatic insufficiency at presentation. Unresponsiveness to nitisinone has already been reported but still the reasons for lack of response have not been clearly identified.[13] Methionine level can be an indicator for non/partial response. There was no significant difference in plasma methionine level between responders (mean, 356.3 μmol/L; range, 13.3–1590 μmol/L) and non/partial-responders at presentation (mean, 465 μmol/L; range, 70–820 μmol/L) but the increased methionine did not decrease to normal (20–50 μmol/L) despite maintenance of adequate nitisinone and Tyr. There was a significant difference between both groups during follow up: mean plasma methionine was 15.9 μmol/L (range, 12–39 μmol/L) in responders, while it was 265 μmol/L (range, 79–470 μmol/L) in non/partial-responders ($P < 0.001$). It is also important to decide when to refer these patients for LT, given that time for recovery from severe liver failure is variable in severely ill patients.[6]

Although nitisinone had significantly improved survival in the present cohort, the overall survival rate of the 36 patients treated with nitisinone was 78%. This rate is higher than in pre-nitisinone reports. van Spronsen *et al*. reported that the natural course of the disease resulted in a mortality rate of 75% for those children diagnosed at <2 months of age by 2 years; 70%, for those diagnosed at 2–6 months of age by 6 years of age; and 40% for those diagnosed at >6 months of age by 10 years of age.[13] Still, the survival rate in the present cohort was lower than in the French and Spanish series, with rates of 97.8% and 100%, respectively.[30,32,33] The most striking difference between the present study and these studies was that nitisinone treatment was initiated after 6 months of age in 69% of the present patients. Temporary interruption of the treatment due to various reasons (e.g. health insurance problems) was common in the present cohort. Permanent interruption of nitisinone treatment resulted in death in three patients. One patient died due to HCC, one patient due to hepatic insufficiency and one because of respiratory insufficiency due to porphyria-like attack. The latest data suggest that early nitisinone treatment was the key factor in the good outcome of the neonatally treated patients, and that a combination of neonatal screening and early nitisinone treatment is recommended.[19–22] But even in early diagnosis patients, drug interruption can lead to increased mortality rate, especially due to HCC.

Liver cancer has been reported as an important risk for patients with HT1 treated with nitisinone.[13,16,33,34] Development of HCC is the main risk for patients with the chronic form or who have been treated with nitisinone after 2 years of age.[33,35] The risk increases if the patient has persistent high AFP and/or has a slow AFP decrease without reaching a normal level.[19,36] In the present cohort, three patients had persistent high AFP along with normal liver function tests, all with proven HCC. Secondary AFP increase was detected in three patients: two with HCC and one with dysplastic changes detected during transplantation. Among these three patients, two had periods of drug interruption of 1–3 months and one was non-compliant to both nitisinone and dietary treatment. The present data support the findings of a previous report that slow decrease of AFP, AFP that never reaches long-standing normalization and secondary increase of AFP are indicative of the development of liver cancer in later life.[19,36] Close follow up of serum AFP, even of minor changes, is important for early detection of HCC and hepatocellular dysplasia especially in late-diagnosis patients. Serial liver ultrasound is also important especially when hypoechoic nodules are detected. All patients with HCC had hypoechoic nodules detected on ultrasound, although malign transformation was not definitely proven on abdominal MRI.

Renal tubular dysfunction with increased echogenicity was common in the present patients, as previously described.[30] The findings resolved with nitisinone treatment although asymptomatic nephrocalcinosis persisted in 4/4 patients. None of the patients developed renal insufficiency during nitisinone treatment.

Episodes of acute neuropathy that clinically resemble porphyric crises occur in up to 50% of untreated children.[36] The mortality is up to 65%.[1,13,37] Competitive inhibition of ALA dehydratase by SA results in a rise in serum ALA and PBG.[38,39] Normalization of SA in blood, correction of the complete inhibition of PBGS in erythrocytes during nitisinone treatment

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protects against porphyric crisis.[14] Schlump *et al*. reported that interruption of nitisinone treatment can cause severe neurological crisis in patients with HT1.[40] In the present cohort, interrupted nitisinone treatment resulted in porphyria-like attacks in three patients, and death in one patient due to respiratory insufficiency.

Although cardiomyopathy has been reported as a frequent finding in HT1, in the present cohort only one patient had septal hypertrophy that responded to nitisinone therapy.[10] Neither the mechanism nor the natural history of this complication is understood. It is suggested that the cardiomyopathy may be due to direct cardiotoxicity of circulating Tyr metabolites during a critical period of vulnerability, and nitisinone reduces the level of these metabolites.[10]

Compliance with dietary treatment is one of the serious problems with nitisinone treatment, especially with increasing age. The recommended level of Tyr, 200–400 μmol/L, is difficult to achieve. Compliance became poorer as the child grew older. Even patients with good and moderate compliance had periods of bad control. The serious dietary non-compliance problems led the parents to prefer LDLT in the present cohort.

Variation of plasma Tyr seems to be an important pathogenic factor in abnormal intellectual development and attention disorder in HT1 patients under long-term treatment with nitisinone.[41–44] In the present cohort, 11 patients had cognitive evaluation and the mean total IQ was 84 (range, 55–115). Only one patient had repeated IQ measurement and a decrease of 3 points was noted. These findings were consistent with recent studies. Bendadi *et al*. noted that the average total IQ score in 10 patients with HT1 receiving nitisinone was significantly lower compared with their unaffected siblings. Repeated IQ measurement in a single-center subset of five patients noted a decline in average IQ score over time.[43] Attention deficit and learning difficulties were common, but behavior was not evaluated in the present cohort.

Lack of gene analysis for the confirmation of diagnosis was a limitation of the present study, and is being addressed in another study.

### *Conclusion*

Nitisinone treatment is effective and improves both the short-term and the long-term prognosis of HT1. Still, early diagnosis on newborn screening is needed because late diagnosis along with delay in treatment carries the risk of persistence of hepatic disease and HCC. In countries where HT1 is not part of newborn screening, it is important to be able to recognize the clinical and laboratory findings in order to prevent delay in diagnosis. More importantly, adding HT1 screening to the nationwide screening program in Turkey is necessary for early diagnosis and facilitation of early treatment, to decrease the mortality and morbidity of the disease both in the short and long term.

Furthermore, multicenter studies in Turkey are needed in order to estimate both the incidence and the clinical course of the disease, with proposed guidelines to improve the long-term outcome of HT1 patients.

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