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CLINICAL RESEARCH ARTICLE

Evaluation of dynamic thiol/disulfide homeostasis in hereditary tyrosinemia type 1 patients

Ayse Cigdem Aktuglu Zeybek [1✉], Ertugrul Kiykim[1], Salim Neselioglu[2], Halise Zeynep Iscan[3], Tanyel Zubarioglu[1], Mehmet Serif Cansever[4] and Ozcan Erel[2]

BACKGROUND: Despite successful treatment with nitisinone, the pathophysiology of long-term complications, including hepatocellular carcinoma and mental decline in tyrosinemia type 1 patients, is still obscure. Oxidative stress may play a role in these complications. While increased fumarylacetoacetate and maleylacetoacetate cause oxidative stress in the liver, increased tyrosine causes oxidative stress in the brain. The aim of this study is to evaluate dynamic thiol/disulfide homeostasis as an indicator of oxidative stress in late-diagnosed tyrosinemia type 1 patients.

METHODS: Twenty-four late-diagnosed (age of diagnosis; 14.43 ± 26.35 months) tyrosinemia type 1 patients (19 under nitisinone treatment and 5 with liver transplantation) and 25 healthy subjects were enrolled in the study. Serum native thiol, total thiol, and disulfide levels were measured, and disulfide/native, disulfide/total, and native thiol/total thiol ratios were calculated from these values.

RESULTS: No significant difference was observed in native, total, and disulfide thiol levels between the groups and no increase in disulfide/native, disulfide/total, and native/total thiol ratios was detected, despite significantly higher plasma tyrosine levels in the nitisinone-treated group.

CONCLUSIONS: We suggest that providing sufficient metabolic control with good compliance to nitisinone treatment can help to prevent oxidative stress in late-diagnosed tyrosinemia type 1 patients.

IMPACT:

- Despite successful nitisinone (NTBC) treatment, the underlying mechanisms of long-term complications in hereditary tyrosinemia type 1 (HT1), including hepatocellular carcinoma and mental decline, are still obscure. Oxidative stress may play a role in these complications.
- Thiol/disulfide homeostasis, which is an indicator of oxidative stress, is not disturbed in hereditary tyrosinemia patients under NTBC treatment, despite higher plasma tyrosine levels and patients who had liver transplantation.
- This is the first study evaluating dynamic thiol/disulfide homeostasis as an indicator of oxidative stress in late-diagnosed HT1 patients.

INTRODUCTION

Hereditary tyrosinemia type 1 (HT1) is a rare autosomal recessively inherited metabolic disorder caused by the impaired catabolism of ʟ-tyrosine (Tyr) due to mutations in the *Fumarylacetoacetate hydrolase* (*FAH*) gene mapped in human chromosome 15q (15q23-25), leading to deficiency in hepatic FAH.[1] The clinical findings are widely heterogeneous even within the same family and usually depend on the onset age of the symptoms.[2] Three clinical phenotypes have been described: an "acute" form (symptoms start within the first 6 months of life with severe liver failure), a "subacute" form (presenting between 6 months and 1 year of age with liver disease, failure to thrive, coagulopathy, hepatosplenomegaly, rickets), and a "chronic" form (symptoms start after 1 year of age with hypophosphatemic rickets, porphyria-like neurologic crisis and chronic liver disease). Cardiomyopathy, hyperinsulinemic hypoglycemia, and pancreatitis are seldomly reported.[2–7] Without treatment, death from liver failure and recurrent bleeding, neurological crisis, or hepatocellular carcinoma (HCC) is inevitable. Nitisinone (NTBC), a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase (4-HPPD, and phenylalanine (Phe) and Tyr-restricted diet are the mainstays of the treatment since 1992, resulting in limitation of liver transplantation indications to severe liver disease unresponsive to NTBC and development of HCC.[3,8–12] Early diagnosis and treatment are important for a better prognosis, because HCC is still the most important long-term complication, especially in late-diagnosed patients.[3,6,8,10–14]

[1]Division of Nutrition and Metabolism, Department of Pediatrics, Cerrahpasa Medical Faculty, Istanbul University-Cerrahpasa, Istanbul, Turkey. [2]Department of Biochemistry, Faculty of Medicine, Yıldırım Beyazıt University, Ankara, Turkey. [3]Department of Pediatrics, Cerrahpasa Medical Faculty, Istanbul University-Cerrahpasa, Istanbul, Turkey. [4]Vocational School of Health Services, Department of Medical Services and Techniques, Istanbul University-Cerrahpasa, Istanbul, Turkey. ✉email: dracaz@iuc.edu.tr

Elevated markers of oxidative damage and depleted antioxidant defenses were reported in various kinds of inborn errors of metabolism (IEM) groups including phenylketonuria, organic acidemias, maple syrup urine disease (MSUD), urea cycle disorders, homocystinuria, glutaric acidemia, and HT1.[15–17] HT1 patients are prone to oxidative stress whether treated with NTBC or not. The enzymatic defect in HT1 leads to the accumulation of toxic compounds fumarylacetoacetate (FAA), maleylacetoacetate (MAA), succinylacetone (SA), and succinylacetoacetate. FAA and MAA have been shown to cause glutathione (GSH) depletion, inhibition of DNA glycosylases, oxidative stress, chromosomal instability, modification in thiol groups, cell cycle arrest, and apoptosis in the cells where it is generated, primarily hepatocytes, suggesting an important mechanism for cytotoxicity, the development of hepatocarcinoma and somatic mosaicism.[18–24] GSH is a major actor of redox homeostasis, which was shown to reduce FAA mutagenicity in cultured cells.[21] GSH depletion due to FAA accumulation is likely to affect DNA repair indirectly as redox homeostasis is also important for this process.[25,26] SA, a competitive inhibitor of δ-aminolaevulinic acid (ALA) dehydratase, leads to the accumulation of ALA that has been associated with mitochondrial toxicity and down-regulation of mitochondrial cytochrome oxidase.[27] NTBC treatment prevents FAA-induced oxidative stress by preventing FAA production in HT1 and even short-term discontinuation of NTBC treatment results in the activation of oxidative stress, GSH metabolism, and liver regeneration pathways.[19] On the other hand, NTBC treatment's most important side effect is Tyr accumulation if not restricted in the diet.[11] Increased Tyr is shown to activate oxidative stress by increasing free radical formation, decreasing brain antioxidant capacity in the cerebral cortex, and inducing DNA damage, as well as altering neurotrophin levels by increasing brain acetylcholinesterase activity.[28–31] Antioxidants have been shown to help reverse changes in the energy metabolism in the rat brain, which was exposed to chronic high Tyr.[30,31]

Thiols are the most vulnerable targets of reactive oxygen species (ROS) and represent a defense system against biochemical disturbances caused by oxidative stress. The transfer of surplus electrons to thiol-including compounds plays an important role in balancing oxidative stress.[32] The thiol-disulfide homeostasis has a dynamic nature. Oxidation of thiol groups causes the generation of disulfide bonds, which is also reversible and can be reduced again to thiol groups. The primary thiols found in plasma are GSH, cysteine, γ-glutamyl cysteine, cysteinyl glycine, and homocysteine.[33] Antioxidant defense, detoxification, signal transduction, regulation of apoptosis, and cellular signaling are cellular processes that are known to be associated with thiol/disulfide homeostasis.[34]

Dynamic thiol/disulfide homeostasis has been studied in only a few inborn errors of metabolism including MSUD and L-2-hydroxyglutaric aciduria.[35,36] Nevertheless, to the best of our knowledge, no study has investigated thiol/disulfide homeostasis in patients with HT1. Therefore, we conducted this study to evaluate thiol/disulfide homeostasis, a novel and sensitive oxidative stress marker, in HT1 children under NTBC treatment or who had liver transplantation (LTx).

MATERIALS AND METHODS
Sample and subjects
Twenty-four HT1 patients in whom the diagnosis was confirmed by detection of urinary succinylacetone excretion, organic acid analysis, and/or molecular analysis of the *FAH* gene were enrolled in the study. The control group consisted of age-matched healthy 25 individuals with no history of chronic disease and/or medication use. Patients who were not under regular follow-up and had poor compliance with NTBC treatment were excluded from the study. Demographic data, including sex and age, were recorded in both HT1 and control groups. Daily NTBC dosage and other medications, laboratory findings including serum transaminases

(alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT)), alfa-fetoprotein (AFP), albumin, urea, creatinine, and daily NTBC dosage were recorded from patients' medical records. The patients were divided into three subgroups according to their presentation time as acute, subacute, and chronic HT1. Five patients had LTx after a long time of NTBC treatment and the remaining 19 patients were still under NTBC treatment at the time of sampling. The patients who were under NTBC treatment were also treated with a protein-restricted diet supplemented with Tyr-free formula.

Plasma samples were collected to determine dynamic thiol/disulfide homeostasis by measuring native thiol (-SH), total thiol (-SH + -S-S-), and thiol-disulfide (−S-S) levels. From these levels, the ratios of disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol were calculated. Simultaneous blood sampling for plasma and quantitative amino acid analysis was performed in both groups. The plasma quantitative amino acid level of the control group was within the normal range.

This prospective study was conducted from January 2018 to September 2018 in the Division of Pediatric Nutrition and Metabolism Unit of Cerrahpasa Medical Faculty. The study protocol was approved by the Ethical Committee of Cerrahpasa Medical Faculty (No.: 83045809-604.01.02-). All participants in both the study and control groups gave informed consent. Informed consent was obtained from the parents of participants younger than 16 years of age.

Plasma preparation
Plasma was prepared from whole blood samples obtained from fasting individuals and HT1 patients by venous puncture using vials with lithium heparin for amino acid analysis and plain tubes for thiol/disulfide homeostasis parameters. Whole blood was centrifuged at 3000 r.p.m.; plasma was removed by aspiration and frozen at −80 °C until determinations. The amino acid analysis was performed by high-pressure liquid chromatography with the use of a Biochrom 30+ series amino acid analyzer (Biochrom 30+, Cambridge, UK).

Thiol/disulfide homeostasis parameters
Serum total thiol and native thiol levels were determined with an automated spectrophotometric method described by Erel and Neselioglu.[33] The principle of the thiol/disulfide measurement method is the reduction of dynamic disulfide bonds ("S" "S") to functional thiol groups ("SH") by sodium borohydride ($NaBH_4$). Disulfide bonds were first reduced to form free functional thiol groups, and unused $NaBH_4$ remnants were completely removed by formaldehyde. The modified Ellman reagent was used to quantify the total thiol content in the samples. The dynamic disulfide amount was calculated by determining half of the difference between the total thiol and the native thiol. After the native thiol (-SH), total thiol (-SH + -S-S-), and disulfide (-S-S) levels were determined, the ratios of disulfide/total thiol, native thiol/ total thiol, and disulfide/native thiol were calculated.

Statistical analysis
Statistical analyses were performed using Statistical Package for Social Sciences version 21.0 (SPSS Inc., Chicago, IL, USA). The mean, standard deviation, median, minimum, maximum, frequency, and ratio values were used as descriptive statistics. The normal distribution of data was evaluated with a Kolmogorov–Smirnov test. For those with normal distribution, parametric tests were used, and data were presented as means ± SD. For variables with abnormal distribution, nonparametric tests were used and data were presented as the median and interquartile range (IQR). The qualitative analysis was performed by using Pearson's χ^2 test and Fisher's exact test. When more than two groups were compared, the nonparametric Kruskal–Wallis test or parametric analysis of variance test was used. The confidence interval was 95% and P values <0.05 were accepted as statistically significant.

RESULTS
Twenty-four late-diagnosed HT1 patients (age of diagnosis; 14.43 ± 26.35 months) who were under regular follow-up and 25 control subjects were enrolled in the study. Ten of the patients were diagnosed as acute (41.7%), eight as subacute (33.3%), and six as chronic HT1 (25%). Eight of the patients were female and 16 patients

Table 1. Biochemical parameters of subtypes of HT1 at the time of sampling.

	Acute HT1 (*n* = 10)	Subacute HT1 (*n* = 6)	Chronic HT1 (*n* = 3)	LTx (*n* = 5)	*P* value
ALT, IU/l (min–max)	21 ± 9.09 (8–33)	31.85 ± 14.96 (19–58)	31 ± 12.19 (21–47)	29.67 ± 7.1 (22–36)	0.319
AST, IU/l median (IQR) (min–max)	25.5 (12) (11–62)	23.5 (40) (19–67)	31 (–) (22–36)	28 (30) (16–67)	0.942
GGT, IU/l (min–max)	15 ± 3.80 (9–20)	17.5 ± 13.5 (2–41)	38.7 ± 24.6 (22–67)	49 ± 49.4 (14–84)	0.159
Albumin, mg/dL (min–max)	4.79 ± 0.30 (4.6-5.4)	4.73 ± 0.544 (4.0–5.4)	4.270 ± 0.44 (3.8–4.7)	4.75 ± 0.22 (4.6-4.9)	0.166
Urea, mg/dL, median (IQR) (min–max)	26 (8.25) (14–45)	28.5 (23.25) (13–37)	23 (–) (11–32)	37 (29.5) (24–61)	0.162
Creatinine, mg/dL, median (IQR) (min–max)	0.5 (0.1) (0.3-0.73)	0.4 (0.57) (0.3–1.06)	0.3 (–) (0.3-0.69)	0.7 (0.51) (0.4–0.8)	0.269
AFP, ng/mL (min–max)	4.94 ± 2.53 (2.2–10.86)	4.83 ± 3.3 (0.6–10.3)	11.75 ± 17.61 (0.86 ± 38)	2.6 ± 0.14 (2.5–2.7)	0.217

Plus or minus values are means ± SD.
Analysis revealed statistically no significant differences between acute, subacute, chronic HT1 patients, and patients with LTx.
AFP alfa-fetoprotein, *ALT* alanine aminotransaminase, *AST* aspartate aminotransferase, *GGT* γ-glutamyl transferase, *IQR* interquartile range, *LTx* liver transplantation.

Table 2. Evaluation of plasma tyrosine and phenylalanine levels of patients with tyrosinemia type 1 under NTBC/low protein diet, patients with liver transplantation, and the control group.

	Patients under NTBC and low protein diet (*n* = 19)	Patients with LTx (*n* = 5)	Control (*n* = 25)	*P* value
Plasma tyrosine level (µmol/l)	469.74 ± 151.199	73.80 ± 28.28	56.37 ± 13.22	**0.000**
Plasma phenylalanine level (µmol/l)	41.10 ± 18.45	56.36 ± 14.609	53.50 ± 12.17	**0.029**

P values in bold are statistically significant.
Plus or minus values are means ± SD.
LTx liver transplantation, *NTBC* nitisinone.

were male. The mean age of the patients was 11.18 ± 7.0 years (min 2.25, max 28.5 years). Eight of the patients were female and 16 were male. Five patients had LTx after NTBC treatment and the remaining 19 patients were still under NTBC treatment at the time of sampling. The patients who were using NTBC were also treated with a Tyr-restricted diet supplemented with a specific formula. No SA excretion was detected in the urine at the time of sampling.

Patients' serum AFP, serum transaminases including ALT, AST, and GGT, urea, and creatinine levels are given in Table 1. No significant difference was detected between HT1 patients under NTBC and low Tyr diet, HT1 patients with LTx.

Plasma Tyr level was significantly higher in HT1 patients under NTBC and low Tyr diet (*p* = 0.000) than both HT1 patients with LTx and the control group. On the other hand, plasma Phe levels were significantly higher in HT1 patients under NTBC and low Tyr diet than in the control group (*p* = 0.032) (Table 2).

The serum thiol/disulfide homeostasis parameters of the groups are shown in Table 3. No significant difference in native thiol (-SH), total thiol (-SH + -S-S-), and disulfide (-S-S) levels was observed between the patients under NTBC treatment, patients with LTx, and control groups (*p* > 0.05). In addition, no significant increase in disulfide/native thiol and disulfide/total thiol ratios was detected in two patient groups (*p* > 0.05) (Table 3). When subtypes of HT1 were considered separately, although acute HT1 patients had the highest native and total thiol levels, no significant differences were found between acute, subacute, and chronic types in thiol/disulfide parameters (Table 4). No correlation was found between serum AFP, ALT, AST, GGT, urea, creatinine, plasma Tyr/Phe, and thiol/disulfide homeostasis markers. Also, no correlation was found between age and thiol/disulfide homeostasis markers.

DISCUSSION

In the present study, we evaluated thiol/disulfide homeostasis, a novel indicator of oxidative stress, in HT1 patients both under NTBC treatment or had LTx after a period of NTBC treatment. No significant differences in native (-SH), total (-SH + -S-S), and disulfide (-S-S) thiol levels were observed among 19 HT1 patients when compared to the healthy control group or LTx patients (Table 2). In addition, no increase in disulfide/native thiol and disulfide/total thiol ratios was determined. There was no correlation between AFP, ALT, AST, GGT, urea, creatinine levels, and thiol/disulfide homeostasis markers. When subgroups of acute, subacute, and chronic HT1 patients were evaluated separately, although a decrease in native and total thiol values was detected, the difference was still not statistically significant. According to these results, we suggested that providing good metabolic control with NTBC treatment may be effective in preventing oxidative stress evaluated by thiol/disulfide homeostasis in HT1 patients despite high Tyr levels.

Living organisms need to convert ROS into more stable molecules to maintain intracellular redox balance, minimize undesirable cellular damage caused by ROS, and sustain life. Various antioxidant mechanisms including enzymes such as superoxide dismutase, catalase, peroxidases like GSH peroxidase, and some nonenzymatic antioxidants such as GSH, NADPH, thioredoxin, vitamin E, vitamin C, selenium, etc. play a role in this inactivation processes.[37] The ideal method is the direct measurement of ROS. However, these are labile compounds even when they are not radicals, and their direct detection is very difficult. Alternative methods of assessing oxidative stress to evaluate changes in "antioxidant status" include measuring the total antioxidant capacity of a biological sample such as a plasma or

Table 3. Comparison of serum thiol/ disulfide homeostasis parameters between HT1 patients under NTBC treatment, patients with liver transplantation, and control subjects.

	HT1 patients (n = 19)	LTx (n = 5)	Control	P value
Native thiol, µmol/l (min–max)	421.15 ± 71.15 (228.8–518.4)	428.2 ± 87.0 (446.5) (309.3–515.9)	419.8 ± 36 (419.4) (356.1–477.6)	0.957
Total thiol, µmol/l (min–max)	459.5 ± 65.1 (461.4) (303.9–560.2)	464.0 ± 86.5 (471.7) (347.6–546.3)	453.6 ± 33.67 (458.1) (398.5–510.2)	0.892
Disulfide, µmol/l (min–max)	19.19 ± 6.3 (19.1) (7.9–37.5)	17.9 ± 4.15 (19.1) (12.6–23.5)	16.88 ± 8.29 (15.6) (4.35–39.25)	0.584
Disulfide/native thiol, % median (IQR) (min–max)	4.49 (2.03) (1.76–16.4)	4.71 (2.79) (2.82–6.2)	3.84 (2.54) (0.91–17.3)	0.697
Disulfide/total thiol, % median (IQR) (min–max)	4.12 (1.71) (1.70–12.36)	4.30 (2.36) (2.67–5.51)	3.34 (2.11) (0.89–8.28)	0.426
Native/total thiol, % median (IQR) (min–-max)	91.76 (3.42) (75.28–96.59)	91.39 (4.72) (88.98–94.66)	93.32 (4.21) (83.43–98.21)	0.426

Plus or minus values are means ± SD.

HT1 hereditary tyrosinemia type 1, *IQR* interquartile range, *LTx* liver transplantation.

tissue sample, measuring free radicals, myeloperoxidase, oxidized proteins, lipids, and thiols, etc.[38]

Oxidative stress has been reported as a risk factor for liver injury in HT1 patients. Previous studies on oxidative stress were related to the tissue level, e.g., liver cells or brain tissue of animals. FAA has been described as a mutagenic, cytostatic, and apoptogenic compound and as a cause of oxidative damage by forming a stable adduct with GSH and protein thiol groups of proteins.[18,21–24,39] The ability of FAA to induce apoptosis due to intracellular GSH depletion appears to be one of the underlying pathologies in the development of acute liver failure in HT1. Accumulation of ALA due to SA also leads to subcellular and tissue damage in the liver.[40] FAA also contributes to genomic instability in HT1 hepatocytes by generating GSH and by being an alkylating agent, leading to increased oxidative stress.[21,23] FAA proves to be an efficient inhibitor of DNA glycosylases that initiate base excision repair, which is the major pathway to remove mutagenic DNA base lesions. The increase in mutagenesis has been proposed as an important mechanism for the development of HCC and somatic mosaicism.[23]

Apoptosis and severe cellular damage have also been described in the kidneys due to local accumulation of FAA. Vacuolization, mitochondrial swelling, lysosomal enlargement at brush borders, compaction, and degeneration of chromatin have been described in proximal renal tubular cells of mouse models.[41,42] Animal model studies show that while FAA causes apoptosis of renal tubular cells, SA mainly impairs tubular solute reabsorption and both can affect gene expression in the kidney.[41,43] Assessment of renal function was not an endpoint in this study. But in our previous study where all patients included in the current study were retrospectively evaluated, mild tubular dysfunction without glomerular involvement was noted before NTBC treatment was initiated. The findings resolved with nitisinone treatment and none of the patients developed renal failure.[44]

Nitisinone is a potent inhibitor of 4-HPPD in the Tyr degradation pathway. 4-HPPD converts 4-hydroxyphenylpyruvate to homo-gentisic acid so that inhibition of the enzyme suppresses the formation of FAA, MAA, and SA. The natural course of the disease, leading inevitably to death from liver failure or HCC, changed after treatment with NTBC. NTBC reversed not only acute liver injury but also progression to chronic liver disease, neuropathic and renal findings in HT1 patients and prevented early development of HCC, especially when started in the neonatal period.[3,6,8,11,12] However, discontinuation of the drug leads to reversal of the symptoms, including acute neuropathic episodes, hepatic dysfunction, and increases the risk of HCC.[11,19,45]

Despite growing evidence that oxidative stress affects the liver and kidneys in untreated HT1 patients, the existence of oxidative stress in NTBC-treated patients remains controversial. Even short-term discontinuation of NTBC therapy resulted in the activation of oxidative stress pathways, GSH metabolism, and liver regeneration in HT1 mice.[19] An increase in Ggt1 and Slc7a11 expression in HT1 mice after NTBC discontinuation leads to an increase in GGT activity due to disrupted transfer of the glutamyl moiety of GSH to a variety of amino acids and dipeptide acceptors and cystine/glutamate antiporter dysfunction that imports cystine as a precursor of GSH synthesis supporting antioxidant responses.[46] A case report revealed that aminopyrine metabolism, related to the cytochrome P450 enzymatic system in liver microsomes, was found normal in an HT1 patient under NTBC treatment. The ketoisocaproic acid metabolism, related to the oxidative capacity of the liver mitochondria, was impaired, expressing that oxidative stress might still affect the HT1 patients under treatment.[47]

NTBC treatment leads to an increase in plasma Tyr levels if not restricted in the diet. Elevated Tyr levels lead to corneal lesions and more importantly to learning difficulties. Increased plasma Tyr level leads to an increase in CSF Tyr concentration and the aim should be to keep the plasma level between 200 and 400 µmol/l, although it is difficult to adjust to dietary treatment[3,6,14] Animal studies showed that high levels of Tyr also cause oxidative stress and DNA damage in the brain and liver.[48,49] Chronic administration of L-Tyr results in a decrease in citrate synthase activity in the hippocampus, striatum, and cerebral cortex of rats and an increase in thiobarbituric acid-reactive species (TBA-RS) levels in the cerebral cortex, a parameter of lipid peroxidation in the cerebral cortex. In addition, the coadministration of n-3 polyunsaturated fatty acid was able to prevent the increase of TBA-RS in the cerebral cortex of these rats, while administration of antioxidant therapy with N-acetylcysteine and deferoxamine was able to prevent the increase in TBA-RS levels and ameliorated the oxidative stress in the rats' brain regions.[48,49] Despite plasma Tyr level was significantly higher in our patients using NTBC therapy, no difference was detected in dynamic thiol homeostasis markers between these patients and the control group. Furthermore, no correlation was detected between plasma Tyr levels and thiol/disulfide homeostasis markers.

Only a few studies have focused on oxidative stress in the long-term follow-up of pediatric patients who received LTx.[50] Hussein et al. revealed that children undergoing LTx for the treatment of inherited metabolic diseases are found to be prone to higher oxidative stress.[51] The authors assessed oxidative stress using the serum oxidative stress index calculated as serum oxidant/

Table 4. Comparison of serum thiol/ disulfide homeostasis parameters between HT1 subgroups and control subjects.

	Acute HT1 ($n = 10$)	Subacute HT1 ($n = 6$)	Chronic HT1 ($n = 3$)	LTx ($n = 5$)	Control ($n = 25$)	P value
Native thiol, µmol/l (min–max)	450.3 ± 40.9 (389.6–510.7)	392.8 ± 100.9 (228.8–518.4)	380.6 ± 58.6 (317.3–432.9)	428.2 ± 87.0 (309.3–515.9)	419.8 ± 36 (356.1–477.6)	0.213
Total thiol, µmol/l (min–max)	486.5 ± 38.7 (426.1–548.9)	436.3 ± 90.0 (303.9–560.2)	416.1 ± 55.28 (354.5–461.5)	464.0 ± 86.5 (347.6–546.3)	453.6 ± 33.67 (398.5–510.2)	0.196
Disulfide, µmol/l (min–max)	18.11 ± 4.8 (7.9–24.6)	21.75 ± 9.33 (8.4–37.5)	17.71 ± 3.1 (14.3–20.2)	17.9 ± 4.15 (12.6–23.5)	16.88 ± 8.29 (4.35–39.25)	0.713
Disulfide/native thiol, % median (IQR) (min–max)	4.17 (1.59) (1.76–6.09)	4.89 (5.46) (2.0–16.4)	5.19 (0) (3.3–5.87)	4.71 (2.79) (2.82–6.2)	3.40 (2.54) (0.91–17.3)	0.742
Disulfide/total thiol, % (min–max)	3.75 ± 1.09 (1.7–5.43)	5.45 ± 3.61 (1.92–12.36)	4.35 ± 1.12 (3.1–5.25)	3.98 ± 1.22 (2.67–5.51)	3.72 ± 1.83 (0.89–8.28)	0.402
Native/total thiol, % (min–max)	92.49 ± 2.17 (89.1–96.6)	89.09 ± 7.22 (75.28–96.16)	91.30 ± 2.23 (89.5–93.81)	92.02 ± 2.46 (88.9–94.6)	92.54 ± 3.66 (83.43–98.21)	0.402

Plus or minus values are means ± SD. HT1: Hereditary tyrosinemia type 1; IQR, interquartile range; LTx: liver transplantation.
HT1 hereditary tyrosinemia type 1, *IQR* interquartile range, *LTx* liver transplantation.

antioxidant ratio, in a total of 16 patients. Two of these patients were HT1. They assumed that these patients would have greater damage to their grafted livers and a higher risk of graft rejection. In our study, five of our patients had LTx and all received living-related LTx. No significant difference in thiol/disulfide homeostasis parameters was observed between the patients with LTx and the control group. Also, no correlation was detected between plasma Tyr level and thiol/disulfide homeostasis markers.

There are some limitations of our study. None of the patients was newly diagnosed and thiol/disulfide homeostasis could not be checked before starting NTBC treatment. Also, none of the patients was early diagnosed by neonatal screening program. The number of patients who had LTx was also limited.

CONCLUSION
To our knowledge, this study is the first study evaluating dynamic thiol/disulfide homeostasis as an indicator of oxidative stress in HT1 patients. Mechanisms underlying HCC development despite treatment and learning difficulties in HT1 patients are still obscure. Oxidative stress has been assumed to play a role in these pathologies depending on previous animal and human studies. Our study group had a large sample size with a number of 25 HT1 patients, 19 under NTBC treatment and 5 on LTx after NTBC treatment. All patients had good compliance to NTBC treatment, but were noncompliant with the Tyr-restricted diet. No evidence of oxidative stress was detected neither during NTBC treatment nor after LTx.

As a result, in our study, we suggest that by providing good metabolic control with regular follow-up and good compliance to NTBC treatment in HT1 patients, it can be possible to diminish oxidative stress evaluated by dynamic thiol homeostasis markers. Further studies with large sample sizes should elucidate the potential factors causing oxidative stress in HT1 patients and may offer different treatment options to prevent liver damage and neurotoxicity besides NTBC treatment and dietary management.

REFERENCES
1. Morrow, G., Angileri, F. & Tanguay, R. M. Molecular aspects of the FAH mutations involved in HT1 disease. *Adv. Exp. Med. Biol.* **959**, 25–48 (2017).
2. van Spronsen, F. J. et al. Hereditary tyrosinemia type I: a new clinical classification with difference in prognosis on dietary treatment. *Hepatology* **20**, 1187–1191 (1994).
3. Mayorandan, S. et al. Cross-sectional study of 168 patients with hepatorenal tyrosinaemia and implications for clinical practice. *Orphanet J. Rare Dis.* **9**, 107 (2014).
4. Baumann, U., Preece, M. A., Green, A., Kelly, D. A. & McKiernan, P. J. Hyperinsulinism in tyrosinaemia type I. *J. Inherit. Metab. Dis.* **28**, 131–135 (2005).
5. Arora, N., Stumper, O., Wright, J., Kelly, D. A. & McKiernan, P. J. Cardiomyopathy in tyrosinaemia type I is common but usually benign. *J. Inherit. Metab. Dis.* **29**, 54–57 (2006).
6. Chinsky, J. M. et al. Diagnosis and treatment of tyrosinemia type I: a US and Canadian consensus group review and recommendations. *Genet. Med.* **12**, 19 (2017).
7. Uçar, H. K., Tümgör, G., Kör, D., Kardaş, F. & Mungan, N. A case report of a very rare association of tyrosinemia type I and pancreatitis mimicking neurologic crisis of tyrosinemia type I. *Balk. Med. J.* **33**, 370–372 (2016).
8. McKiernan, P. J., Preece, M. A. & Chakrapani, A. Outcome of children with hereditary tyrosinaemia following newborn screening. *Arch. Dis. Child.* **100**, 738–741 (2015).
9. Holme, E. & Lindstedt, S. Nontransplant treatment of tyrosinemia. *Clin. Liver Dis.* **4**, 805–814 (2000).
10. Seda Neto, J. et al. HCC prevalence and histopathological findings in liver explants of patients with hereditary tyrosinemia type 1. *Pediatr. Blood Cancer* **61**, 1584–1589 (2014).
11. van Ginkel, W. G. et al. Long-term outcomes and practical considerations in the pharmacological management of tyrosinemia type 1. *Paediatr. Drugs* **21**, 413–426 (2019).
12. Bartlett, D. C., Lloyd, C., McKiernan, P. J. & Newsome, P. N. Early nitisinone treatment reduces the need for liver transplantation in children with tyrosinaemia type 1 and improves post-transplant renal function. *J. Inherit. Metab. Dis.* **37**, 745–752 (2014).
13. Giguère, Y. & Berthier, M. T. Newborn screening for hereditary tyrosinemia type I in Québec: update. *Adv. Exp. Med. Biol.* **959**, 139–146 (2017).
14. de Laet, C. et al. Recommendations for the management of tyrosinaemia type 1. *Orphanet J. Rare Dis.* **8**, 8 (2013).
15. Ercal, N., Aykin-Burns, N., Gurer-Orhan, H. & McDonald, J. D. Oxidative stress in a phenylketonuria animal model. *Free Radic. Biol. Med.* **32**, 906–911 (2002).
16. Mc Guire, P. J., Parikh, A. & Diaz, G. A. Profiling of oxidative stress in patients with inborn errors of metabolism. *Mol. Genet. Metab.* **98**, 173–180 (2009).
17. Guerreiro, G. et al. Oxidative damage in glutaric aciduria type I patients and the protective effects of l-carnitine treatment. *J. Cell. Biochem.* **119**, 10021–10032 (2018). 12.
18. Tanguay, R. M., Angileri, F. & Vogel, A. Molecular pathogenesis of liver injury in hereditary tyrosinemia 1. *Adv. Exp. Med. Biol.* **959**, 49–64 (2017).
19. Colemonts-Vroninks, H. et al. Oxidative stress, glutathione metabolism, and liver regeneration pathways are activated in hereditary tyrosinemia type 1 mice upon short-term nitisinone discontinuation. *Genes.* **12**, 3 (2020).
20. Lloyd, A. J., Gray, R. G. & Green, A. Tyrosinaemia type 1 and glutathione synthetase deficiency: two disorders with reduced hepatic thiol group concentrations and a liver 4-fumarylacetoacetate hydrolase deficiency. *J. Inherit. Metab. Dis.* **18**, 48–55 (1995).

21. Jorquera, R. & Tanguay, R. M. The mutagenicity of the tyrosine metabolite, fumarylacetoacetate, is enhanced by glutathione depletion. *Biochem. Biophys. Res. Commun.* **232**, 42–48 (1997).

22. Jorquera, R. & Tanguay, R. M. Fumarylacetoacetate, the metabolite accumulating in hereditary tyrosinemia, activates the ERK pathway and induces mitotic abnormalities and genomic instability. *Hum. Mol. Genet.* **10**, 1741–1752 (2001).

23. Bliksrud, Y. T., Ellingsen, A. & Bjørås, M. Fumarylacetoacetate inhibits the initial step of the base excision repair pathway: implication for the pathogenesis of tyrosinemia type I. *J. Inherit. Metab. Dis.* **36**, 773–778 (2013).

24. Edwards, S. W. & Knox, W. E. Homogentisate metabolism: the isomerization of maleylacetoacetate by an enzyme which requires glutathione. *J. Biol. Chem.* **220**, 79–91 (1956).

25. Langie, S. A. et al. The role of glutathione in the regulation of nucleotide excision repair during oxidative stress. *Toxicol. Lett.* **168**, 302–309 (2007).

26. Storr, S. J., Woolston, C. M. & Martin, S. G. Base excision repair, the redox environment and therapeutic implications. *Curr. Mol. Pharm.* **5**, 88–101 (2012).

27. O'Brien, P. & Lee, O. *Modifications of Mitochondrial Function by Toxicants* 2nd edn, Vol. 1 (Elsevier, 2010).

28. Sgaravatti, A. M. et al. Tyrosine promotes oxidative stress in cerebral cortex of young rats. *Int. J. Dev. Neurosci.* **26**, 551–559 (2008).

29. De Prá, S. D. et al. L-Tyrosine induces DNA damage in brain and blood of rats. *Neurochem. Res.* **39**, 202–207 (2014).

30. Teodorak, B. P. et al. Antioxidants reverse the changes in energy metabolism of rat brain after chronic administration of L-tyrosine. *Metab. Brain Dis.* **32**, 557–564 (2017). 04.

31. Langlois, C. et al. Rescue from neonatal death in the murine model of hereditary tyrosinemia by glutathione monoethylester and vitamin C treatment. *Mol. Genet. Metab.* **93**, 306–313 (2008).

32. Baba, S. P. & Bhatnagar, A. Role of thiols in oxidative stress. *Curr. Opin. Toxicol.* **7**, 133–139 (2018).

33. Erel, O. & Neselioglu, S. A novel and automated assay for thiol/disulfide homeostasis. *Clin. Biochem.* **47**, 326–332 (2014).

34. Biswas, S., Chida, A. S. & Rahman, I. Redox modifications of protein-thiols: emerging roles in cell signaling. *Biochem. Pharm.* **71**, 551–564 (2006).

35. Cansever, M. S. et al. Oxidative stress among L-2-hydroxyglutaric aciduria disease patients: evaluation of dynamic thiol/disulfide homeostasis. *Metab. Brain Dis.* **34**, 283–288 (2019).

36. Zubarioglu, T. et al. Evaluation of dynamic thiol/disulphide homeostasis as a novel indicator of oxidative stress in maple syrup urine disease patients under treatment. *Metab. Brain Dis.* **32**, 179–184 (2017).

37. Rahal, A. et al. Oxidative stress, prooxidants, and antioxidants: the interplay. *Biomed. Res. Int.* **2014**, 761264 (2014).

38. Lemineur, T., Deby-Dupont, G. & Preiser, J. C. Biomarkers of oxidative stress in critically ill patients: what should be measured, when and how. *Curr. Opin. Clin. Nutr. Metab. Care* **9**, 704–710 (2006).

39. Dieter, M. Z. et al. Pharmacological rescue of the 14CoS/14CoS mouse: hepatocyte apoptosis is likely caused by endogenous oxidative stress. *Free Radic. Biol. Med.* **35**, 351–367 (2003).

40. Cardoso, V. E. S. et al. Liver damage induced by succinylacetone: a shared redox imbalance mechanism between tyrosinemia and hepatic porphyrias. *J. Braz. Chem. Soc.* **28**, 1297–1307 (2017).

41. Sun, M. S. et al. A mouse model of renal tubular injury of tyrosinemia type 1: development of de Toni Fanconi syndrome and apoptosis of renal tubular cells in Fah/Hpd double mutant mice. *J. Am. Soc. Nephrol.* **11**, 291–300 (2000).

42. Grompe, M. The pathophysiology and treatment of hereditary tyrosinemia type 1. *Semin. Liver Dis.* **21**, 563–571 (2001).

43. Maiorana, A. & Dionisi-Vici, C. NTBC and correction of renal dysfunction. *Adv. Exp. Med. Biol.* **959**, 93–100 (2017).

44. Zeybek, A. C. et al. Hereditary tyrosinemia type 1 in Turkey: twenty year single-center experience. *Pediatr. Int.* **57**, 281–289 (2015).

45. Önenli Mungan, N. et al. Tyrosinemia type 1 and irreversible neurologic crisis after one month discontinuation of nitisone. *Metab. Brain Dis.* **31**, 1181–1183 (2016). 10.

46. Lewerenz, J. et al. The cystine/glutamate antiporter system x(c)(-) in health and disease: from molecular mechanisms to novel therapeutic opportunities. *Antioxid. Redox Signal.* **18**, 522–555 (2013).

47. Rigante, D., Gasbarrini, A., Nista, E. C. & Candelli, M. Decreased mitochondrial oxidative capacity in hereditary tyrosinemia type 1. *Scand. J. Gastroenterol.* **40**, 612–613 (2005).

48. Carvalho-Silva, M. et al. Omega-3 fatty acid supplementation decreases DNA damage in brain of rats subjected to a chemically induced chronic model of Tyrosinemia type II. *Metab. Brain Dis.* **32**, 1043–1050 (2017).

49. Streck, E. L. et al. Role of antioxidant treatment on DNA and lipid damage in the brain of rats subjected to a chemically induced chronic model of tyrosinemia type II. *Mol. Cell. Biochem.* **435**, 207–214 (2017).

50. Hussein, M. H. et al. Oxidative stress after living related liver transplantation subsides with time in pediatric patients. *Pediatr. Surg. Int.* **27**, 17–22 (2011).

51. Hussein, M. H. et al. Children undergoing liver transplantation for treatment of inherited metabolic diseases are prone to higher oxidative stress, complement activity and transforming growth factor-β1. *Ann. Transplant.* **18**, 63–68 (2013).

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The authors declare no competing interests.

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The research was conducted according to the World Medical Association Declaration of Helsinki and each subject signed informed consent before participating in the study.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Ayse Cigdem Aktuglu Zeybek.

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