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Phenotype, genotype, and outcome of 25 Palestinian patients with hereditary tyrosinemia type 1

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ABSTRACT

Background: Tyrosinemia type 1 (hepatorenal tyrosinemia, HT1) is a rare autosomal recessive inborn error of tyrosine metabolism caused by deficiency of the last enzyme in the tyrosine catabolic pathway, fumarylacetoacetate hydrolase (FAH) leading to severe hepatic, renal and peripheral nerve damage if left untreated. Early treatment may prevent acute liver failure, renal dysfunction, liver cirrhosis, hepatocellular carcinoma (HCC) and improves survival.

Material and methods: A retrospective single center study was carried out based on the clinical and biochemical presentation, therapy and outcome of 25 Palestinian patients with HT1 diagnosed during the last 25 years.

Results: HT1 is not included in newborn screening program in Palestine. The mean age at diagnosis was 8 months and the main clinical manifestations were coagulopathy, hepatomegaly, splenomegaly and renal tubular dysfunction. The main biochemical abnormalities were elevated plasma tyrosine, serum transaminases and prothrombin time, and low serum phosphorous with elevated alkaline phosphatase compatible with hypophosphatemic rickets secondary to renal tubular dysfunction. All patients were treated with nitisinone. The mean duration of nitisinone treatment was 74 months and the mean dosage was 0.89 mg/kg/day. None developed HCC or neurological crisis.

Conclusions: Most patients present with liver failure and renal tubular dysfunction. Nitisinone treatment was effective therapy in all patients and improved both short- and long-term prognosis of HT1. Renal tubular dysfunction improved in all patients within the first week of starting nitisinone. Early diagnosis is necessary because delay in the treatment increases the risk of progressive liver failure HCC, progressive renal disease and neuropathy.

1. Introduction

Tyrosinemia type 1 (hepatorenal tyrosinemia, HT1) is a rare autosomal recessive heterogeneous multisystemic genetic disease (OMIM 276700) caused by deficiency of fumarylacetoacetate hydrolase (FAH), the terminal enzyme in tyrosine metabolism [1]. FAH is highly expressed in hepatocytes and renal tubular cells. Blockage leads to accumulation of the toxic metabolites fumarylacetoacetate (FAA), maleylacetoacetate (MAA), succinylacetoacetate (SAA), and succinylacetone (SA), which play a major role in the pathogenesis of the clinical manifestations and complications of the disease including progressive hepatorenal disease and neurological findings [2]. 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. Clinical symptoms typically begin before the age of 2 years with the majority of children presenting with acute liver failure and renal dysfunction [3]. Other clinical manifestations include neurological crisis, hypophosphatemic rickets and hypertrophic cardiomyopathy [3,4]. Silent tyrosinemia without elevated tyrosine and succinylacetone has been reported [5]. All survivors are at an extremely high life time risk of developing hepatocellular carcinoma (HCC). The natural history of the disease usually results in death if left untreated. Liver transplantation (LT) was the first definitive therapy introduced. Low Tyrosine-phenylalanine diet was not effective in the acute form and had limited success in the chronic form, nor did it prevent

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HCC.

Treatment with Nitisinone (2-Nitro-4-trifluoromethylbenzoyl)-1,3- cyclohexanedione (NTBC), a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase, leading to the suppression of MAA, FAA and SA accumulation has dramatically changed the outcome of the disease [6,7]. It had significantly improved survival in HT1 and decreased the risk of early development of HCC in those who began treatment at an early age. It is also an efficient drug for treatment of renal tubular dysfunction allowing a rapid improvement of kidney function [6–8]. Intellectual impairment has been proposed as a complication of hypertyrosinemia under NTBC treatment [9].

In the present study we reported the genotype, clinical and biochemical features, and outcome of 25 Palestinian patients with HT1. The aim was to increase the knowledge on clinical outcome in these patients, and improve understanding of current diagnosis and clinical management practices for HT1 in countries where expanded newborn screening is not part of the newborn screening program.

2. Material and Methods

We reviewed all patients with HT1 diagnosed in the Metabolic department, Makassed charitable Hospital, Jerusalem in the period from January 1992 to June 2017. A detailed history was taken from all patients or their families. Clinical examination and laboratory evaluation including full blood count, liver transaminases, kidney function tests, coagulation profile, serum calcium, phosphate, alkaline phosphatase (ALP), alpha fetoprotein (AFP), plasma quantitative amino acid analysis, urine qualititative organic acid analysis and abdominal ultrasound were performed at the time of presentation and during follow up at the metabolic clinic. The diagnosis of HT1 was made on the basis of detection of elevated SA in urine samples (qualitative) and elevated plasma tyrosine, and confirmed by molecular genetic analysis in all patients. All patients were treated with tyrosine and phenylalanine-restricted diet under the supervision of a clinical dietician. NTBC was immediately started after the diagnosis was confirmed by elevated SA in urine by Gas Chromatography Mass Spectrometry (GC-MS).

3. Results

The Clinical and biochemical data of twenty-five HT1 patients from 10 families (10 females, 15 males) aged between 1 month and 25 months at the time of diagnosis were studied. 23 patients (92%) were born to consanguineous parents. The mean age at diagnosis was 8 months. 3 patients (12%) had very early onset of symptoms (below 2 months), 9 patients (36%) had early onset (between 2 and 6 months) and 13 patients (52%) had late onset (more than 6 months). The main clinical and biochemical findings were coagulopathy which has been reported in 20 patients (80%), hepatomegaly in 19 patients (76%), splenomegaly in 16 patients (64%) and renal disease in the form of enlarged kidneys and increased echogenicity in 14 patients (56%). 12 patients (48%) had clinical and radiological evidence of rickets caused by hypophosphatemia secondary to proximal renal tubular dysfunction. Jaundice was seen in 7 patients (28%) (Fig. 1).

Lab results were plotted against age specific normal values. They were performed on presentation and during follow up at regular intervals. Partial thromboplastin time was elevated in 14 patients (56%), Prothrombin time and INR in 20 patients (80%), alanine amino transferase in 11 patients (44%), aspartate transaminase in 22 (88%), and alkaline phosphatase in 19 patients (76%).

24 patients (96%) had elevated serum α-fetoprotein (AFP) level for age, elevated serum tyrosine level and increased concentration of succinylacetone in urine using Gas chromatography-Mass spectrometry (qualitatively).

Serum phosphorus was low in 14 patients (56%), 12 of them (48%) had clinical and radiological evidence of rickets, normal in 7 patients (28%) and was not reported in 4 patients (16%) at the time of diagnosis (Table 1). Tubular reabsorption of phosphorous in 24-h urine collection and Glomerular filtration rate were not measured.

Molecular genetic analysis of *FAH* revealed 7 previously reported mutations, the most common being reported in 14 patients from a single district in southern west bank was the mis-splicing IVS8-1 G > C mutation leading to the loss of 51 residues in the protein (Table 2). The parents of all these patients were heterozygous for the mutation and all sibs were either heterozygous or normal homozygotes.

Abdominal ultrasound was performed at the time of the diagnosis in 20 patients, of whom 50% showed abnormalities in form of hepatomegaly, coarsened hepatic echotexture and increased nodularity. One patient had hypoechoic focal lesion about 7 mm in diameter that did not increase in size at follow-up visits and did not show signs of malignancy in abdominal CT scan. Standard laboratory investigations and abdominal ultrasound were also recorded at each follow-up visit. Two patients developed portal hypertension. Liver biopsy was performed in two patients due to persistent elevation of serum transaminases revealing features of cirrhosis in one patient and features of fibrosis in the other. None of our patient developed hepatic tumor or neurologic crisis.

Treatment included low-protein diet, special dietary formula free of tyrosine and phenylalanine (Tyros, Med Jonson). NTBC was started immediately after the diagnosis was confirmed by urine organic acid analysis revealing elevated succinylacetone, and plasma amino acid analysis revealing elevated tyrosine. The recommended starting dose was 1 mg/kg/day, but the actual dose ranged from 0.44 to 1 mg/kg/day (mean 0.89 mg/kg/day). The reason for the variation in the dose was due to treatment interruption because of high cost of therapy and decreased availability through the Palestinian Ministry of Health. The mean duration of therapy was 74 months, and the age at the start of NTBC ranged from 5 months to 13 years.

All patients had dramatic initial response to NTBC by means of normalization of all laboratory parameters including coagulopathy, serum aminotransferase and AFP. Treatment resulted also in rapid normalization of serum phosphate within few days followed by resolution of rachitic changes within the first month. No difference was noted between patients who received drug dose below 1 mg/kg/day and those who received a dose equal or above 1 mg/kg/dose.

Plasma tyrosine was recorded at follow-up visits in 20 patients and was below 500 μmol/L in 19 patients indicating adequate compliance with dietary advice. Complications related to poor compliance to dietary advice were reported in 4 patients (17.3%) and included excessive tearing and eye dryness in 3 patients and pseudo dendritic keratitis in 1 patient. Neurological examination revealed normal results regarding motor, language and behavior but the patients were not tested with Bayley Scales of Infant Development or other standardized intelligence tests.

Survival rate of patients on NTBC therapy was 92%. Two patients died due to liver failure after interruption of therapy.

4. Discussion

HT1 is not included in newborn screening program in Palestine. The present study is the first to describe the phenotype/genotype and long-term outcome of HT1 in Palestine. Metabolic disorders as

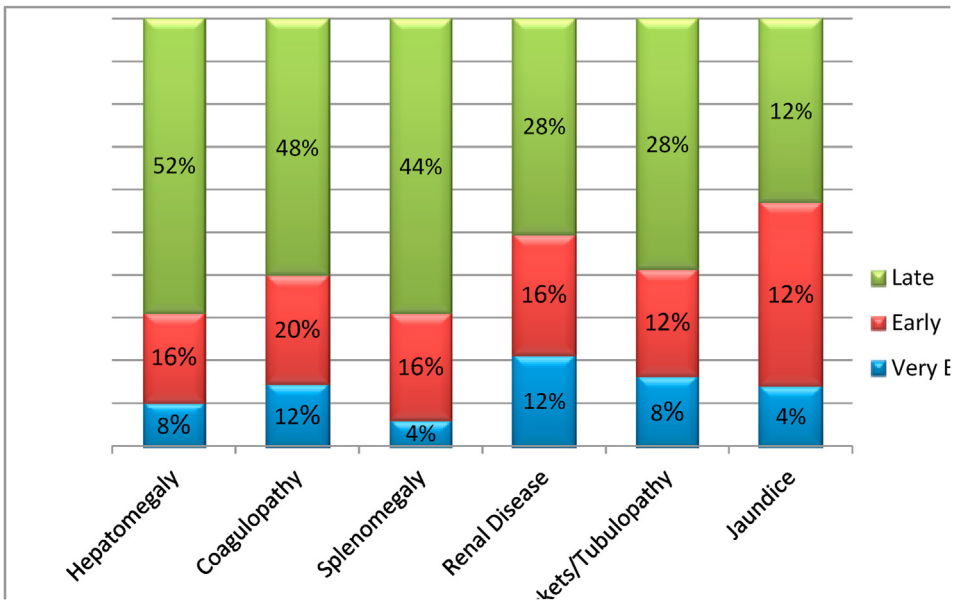

Fig. 1. Common manifestations and the age of presentation of patients with HT1.

Table 1
Laboratory findings of the patients with hereditary tyrosinemia type 1 at the onset of symptoms.

Laboratory Test	N o rmal values	Mean value Very Early onset (n = 5)	Mean value Early onset (n = 7)	Mean value Late onset (n = 13)
Hemoglobin (g/dL)	10.5–12	10.07	10.96	10.81
WBC (cell/mL)	5–16	14.17	10.74	11.13
Platelets (per mL)	150–400	229	123	149
Creatinine (mg/dL)	0.3–0.8	46 0	0.34	0.33
Blood urea nitrogen (mg/dL)	0.2–0.7	7.52	6.20	9.13
Partial thromboplastin time (sec)	150–500	68.75	74.80	45.83
Prothrombin time (sec)	11–15	20.37	36.40	21.49
INR	0.9–1.4	3.40	4.50	2.11
Phosphorous (mg/dL)	3.8–6.5	4.10	2.94	3.27
Calcium (mg/dL)	8.5–10.5	10.73	9.62	9.23
Alanine aminotransferase (units/L)	<40	35.25	77.60	45.04
Aspartate aminotransferase (units/L)	<60	76.50	97.40	106
Total serum bilirubin (g/dL)	<1	2.10	3.02	1.95
Gamma glutamyltransferase (units/L)	0–30	170	190	198
Glucose (mg/dL)	60–105	104	80	98
Alkaline Phosphatase (Units/L)	60–200	982	2721	1574
Albumin (g/dL)	2.6–4.2	3.55	3.53	3.91
Total Protein (g/dL)	4.1–8	5.25	5.23	6.29
Alpha-fetoprotein (ng/mL)	0–100	552975	195050	47341
Phenylalanine (μmol/L)	26–95	111	245	67
Tyrosine (μmol/L)	24–115	376	366	315

Table 2
Genotype of the patients with hereditary tyrosinemia type 1.

The Mutation in *FAH* gene	Number of Patients
IVS8-1 G > C Homozygous	14
P261L (C > T) Homozygous	2
Q64H (G > T) Homozygous	1
IVS6-1 G > T Homozygous	2
IVS12 + 5 (G > A) Homozygous	4
I121T (T→C) & IVS12 + 5 (G→A) Compound Heterozygous	1
R341P Homozygous	1

well as other inherited disorders are relatively common among Palestinian people due to high rate of consanguineous marriage. The rate of consanguinity was (92%), higher than in Egypt, Turkey, and Spain [1,2]. 12% had onset of symptoms below the age of 2 months, 36% presented between 2 and 6 months of age and 52% presented after age 6 months. The mean age at diagnosis was 8 months. In a previous study, the mean age at onset of symptoms was 9 months [1], and in another study, the mean age at diagnosis was 4.25 months [2].

In the present study, the acute form constituted the majority of cases. The main clinical manifestations were hepatomegaly, coagulopathy, and splenomegaly while jaundice was present in only 28% of patients. The coagulopathy was more common relative to elevated serum transaminases and serum bilirubin. Cardiac echocardiogram was not performed and none had signs of cardiac disease [4]. Silent tyrosinemia type I has been described in a family with three affected children who developed HCC after age 11 years

secondary to unexplained hepatosplenomegaly and cirrhosis during infancy. Succinylacetone and tyrosine were normal. Whole exome sequencing (WES) identified a novel homozygous c.424A > G (p.R142G) in exon 5 of *FAH* gene [5]. In contrast to normal succinylacetone and tyrosine in this report, all patients in our series had elevated urine succinylacetone and plasma tyrosine at the time of the diagnosis.

Before the introduction of NTBC, 17–37% of affected children develop HCC. Similarly, tubular dysfunction produced chronic renal disease, hypopphosphatemic rickets and renal tubular acidosis and porphyria-like crisis [3,6–8].

Few studies describing long-term outcome of NTBC in HT1 are available. They concluded that it greatly improved survival and quality of life, has rapid early effect on renal tubular cells by normalizing plasma phosphate and GFR, and no glomerular filtration abnormality was detected [6–8], but some worrying items were observed as persistent elevation of alpha-fetoprotein, coarse hepatic architecture, cirrhosis and HCC [3,6].

In the present study, NTBC resulted in dramatic improvement in renal tubular dysfunction which was maintained during follow up visits evidenced by normal serum phosphorous and resolution of signs of rickets. 2 patients died after they discontinued treatment and lost to follow up making the survival rate 92%. However, 4 patients developed hepatic complications; one patient was diagnosed at age 2 years and developed liver cirrhosis at age 13 years. Another patient was diagnosed at age 3 years and developed hepatic fibrosis at age 12 years. Two patients were diagnosed at age 6 and 8 months, developed portal hypertension at age 4 and 5 years respectively. Hepatomegaly, coagulopathy and renal tubular dysfunction were the main clinical features in all 4 patients, but no genotype-phenotype correlation was noted.

Another long-term complication of NTBC is the development of intellectual impairment secondary to elevated plasma tyrosine similar to other causes of hypertyrosinemia. In one study, five out of seven patients (71%) above 3 years performed below average on standardized intelligence tests (SON-R, K-ABC) [9]. A high number of patients with HT1 were found in another study to perform below normal in the assessment of psychomotor development and intellectual functioning [10]. None of our patients develop neurologic crisis before and after starting NTBC [11].

The most prevalent mutation in the present study reported in 14 patients was homozygosity for IVS8-1 G > C and was prevalent in southern West Bank which is explained by founder effect.

We also identified homozygosity for other mutations commonly reported in other ethnic groups and geographic locations. These included IVS12+5G > A in 4 patients. This mutation is prevalent in the French-Candian population mainly in Quebec [3]. The IVS6-1G > T (c.554-1G > T) was recorded in 2 patients. This mutation is the most prevalent mutation in southern Europe [2,3]. The third mutation is 782C- > T substitution in exon 9, changing proline at codon 261 to leucine (Pro261Leu) in 2 patients. It was found in Ashkenazi Jews [12]. The high concentration of certain mutations in these populations and geographic locations is most probably explained by founder effect. No genotype/phenotype correlation was noted with these mutations.

Liver imaging should be carried out periodically for early detection of HCC. In one series, imaging features were recorded in 16 patients with HT1. 13 patients (81%) had multiple liver nodules. 4 patients had HCC [13]. Only one patient in our series had liver nodule that did not show signs of malignancy in abdominal CT scan.

5. Conclusion

Early initiation of NTBC improves both short and long-term prognosis of patients with HT1 especially the acute liver failure and renal tubular dysfunction. In our study, we did not perform quantitative SA level in blood or urine but no difference in clinical and laboratory parameters was noted between patients taking 1 mg/kg/day or higher and those taking lower doses, but further studies are needed to determine the optimal dose in patients with HT1. We did not record genotype/phenotype correlation in the reported mutations.

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CRediT authorship contribution statement

Imad Dweikat: Conceptualization, Methodology, Writing - original draft. **Nada Qawasmi:** Data curation, Formal analysis. **Aysha Najeeb:** Data curation. **Mohammad Radwan:** Data curation.

Declaration of competing interest

None of the authors has any conflict of interest to disclose.

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