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PRISMA statement of preferred reporting items for systematic reviews and meta-analyses (Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 2009; 6(7): e1000097.) (http://www.prisma-statement.org/);

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Acknowledgements: Any technical or financial support or editorial contributions (statistical analysis, English evaluation) towards the study should appear at the end of the article.

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Editorial

Dear JPR readers,

We are so proud and happy to welcome you to the special inherited metabolic diseases issue of *"The Journal of Pediatric Research"*.

Inherited metabolic diseases are mostly inherited and occasionally *de novo* genetic disorders of the biosynthesis or breakdown of substances within specific pathways that were recognised by specific biochemical tests and sometimes treatable by metabolic intervention. In spite of known as "rare" disease, inborn error of metabolism, cumulatively affect approximately one in every 500 newborns. Therefore, they represent a special challenge in general and pediatric practice.

In the era of revolution in enzymatic and genetic diagnosis of inborn metabolic diseases this special issue is coming to illustrate and discuss the experience with enzymatic diagnosis in lysosomal storage disease, clinical findings and treatment in patients with Tyrosinemia Type I and different types of Mucopolysaccharidosis (Type II and IV). The readers will find the extensive knowledge for broad spectrum of inherited metabolic disease: from GM2- gangliosidosis, Niemann-Pick A and B, L-2-Hydroxyglutaric aciduria to Alkaptonuria. Interesting case reports will demonstrate the challenges in the diagnosis of patients with inherited metabolic disease: Glutaric aciduria Type I case with normal glutaryl carnitine, co-existence of two inherited metabolic disease in two patients and siblings with very rare disease: Ethylmalonic aciduria.

We would like to express deep gratitude to our authors, reviewers, editorial board members, Galenos Publishing House Officers and Nobel Pharmacy for their hard work in creating and support of this special issue.

Have a nice reading,

Best wishes

İzmir, May 2018

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Original Article

Clinical Features of 29 Patients with Hereditary Tyrosinemia I in Western Turkey

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ABSTRACT

Aim: The aim of this study was to investigate the long-term outcome of hereditary tyrosinemia Type I (HTI) patients treated with 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) to increase knowledge about the clinical outcome in these patients. We want to mention that the patients with HTI have heterogeneous clinic. Early diagnosis and early treatment important to prevent the complications.

Materials and Methods: A retrospective study was carried out with twenty nine patients with HTI and who had been followed up by Ege University Faculty of Medicine, Department of Pediatric Metabolic Diseases and Nutrition Unit between December 1996 and September 2017.

Results: Eight patients were acute form, thirteen were subacute and eight patients were chronic form. Mean age onset of clinical symptoms was 3.7±1.6, 9±1.6 and 41±27 months in acute, subacute and chronic HTI patients, respectively. The mean interval from the first symptom the diagnosis was 12.2 months. Mean of follow-up was 82.2 months (minimum: 1 month-maximum: 203 months). Five patients of HTI diagnosed with hepatocellular carcinoma and neurogenic crises were detected in four patients.

Conclusion: NTBC treatment is effective and improves the prognosis of HTI. But early diagnosis and treatment leads to much better outcome. Adherence to the diet and treatment and follow-up schedule of the patients are vital.

Keywords: Tyrosinemia Type I, nitisinone, hepatocellular carcinoma, neurogenic crises, Turkey

Introduction

Hereditary tyrosinemia Type I (HTI, OMIM 276700) is a rare inborn error of tyrosine metabolism due to deficiency of the enzyme fumarylacetoacetate hydrolase (FAH), the last enzyme in the tyrosine catabolic pathway (1) (Figure 1). Biochemically, patients typically have hypertyrosinemia and toxic metabolites. Toxic metabolites and their derivates such as fumarylacetoacetate (FAA), maleylacetoacetate, succynylacetoacetate (SA) and SA playa major role in tissue damage with hepatic, renal and neurological findings (2). The *FAH* gene is mapped in human chromosome 15q (15q23-25) and consists of 14 exons spanning over 35 kb of DNA. Up to now, approximately 100 mutations in the *FAH* gene have been associated with HTI and the mutations are listed in the Human Genome Mutation database (HGMD® Professional 2016.1, accessed on April 2016) (3,4).

The clinic symptoms of hereditary HTI are numerous even within the same family members and can appear in any term extending from neonatal period to adulthood. Clinically, tyrosinemia Type I may be classified based on the age at the onset of symptoms, which broadly correlates with disease severity. The first form is an acute form that manifests before six months of age with acute liver failure but rarely in the first two weeks of life. The second form is a subacute form presenting between six months and one year of age with liver disease, failure to thrive, coagulopathy, hepatosplenomegaly, rickets and hypotonia. The last form is a chronic form that presents after the 1st year with chronic liver disease, renal disease, rickets, cardiomyopathy and/or a porphyria-

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like syndrome (1,5). Porphrya-like syndrome, in other words neurogenic crises, is usually precipitated by intercurrent infection or interruption of 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione NTBC. The crises, which may be severe, are characterised initially by pain (including abdominal pain mimicking an acute surgical emergency), weakness and autonomic changes such as hypertension and hyponatremia. Patients may develop an acute progressive ascending motor neuropathy and convulsion.

There is a hepatocellular carcinoma (HCC) development risk (1). Other clinical manifestations that less frequently observed are hypoglycemia due to hyperinsulinism (6) and hypertrophic cardiomyopathy (7,8).

For the diagnosis, SA is the most useful test that may be measured in urine, plasma and dried blood spot. Even though, very rarely, urine SA elevation may be absent in mild cases (9). Newborn screening with SA from dried blood spots has been established in some countries. In this manner, the treatment to be started before the development of clinical symptoms improves the prognosis of disease (10,11). Mutation analysis of FAH gene provides confirmation of the diagnosis and antenatal diagnosis (12). The phenylalanine and tyrosine restricted diet and nitisinone NTBC are base in the treatment of HTI. There are two aims in treatment of HTI; diet is for reduction of tyrosine levels and NTBC is for inhibition of formation of toxic metabolites (1,13). Point action of NTBC is the second step of tyrosine catabolic pathway by inhibiting 4-hydroxyphenylpyruvate dioxygenase (14) (Figure 1). It is important to commence nitisinone at an early period and comply with the follow-up and treatment. HCC development risk has reduced since nitisinone came into use (15). The aim of this study was to investigate the long-term outcome of HTI patients treated with NTBC to increase knowledge about the clinical outcome in these patients. We want to mention that the patients with HTI have heterogeneous clinic. Early diagnosis and early treatment are substantial to prevent the complications.

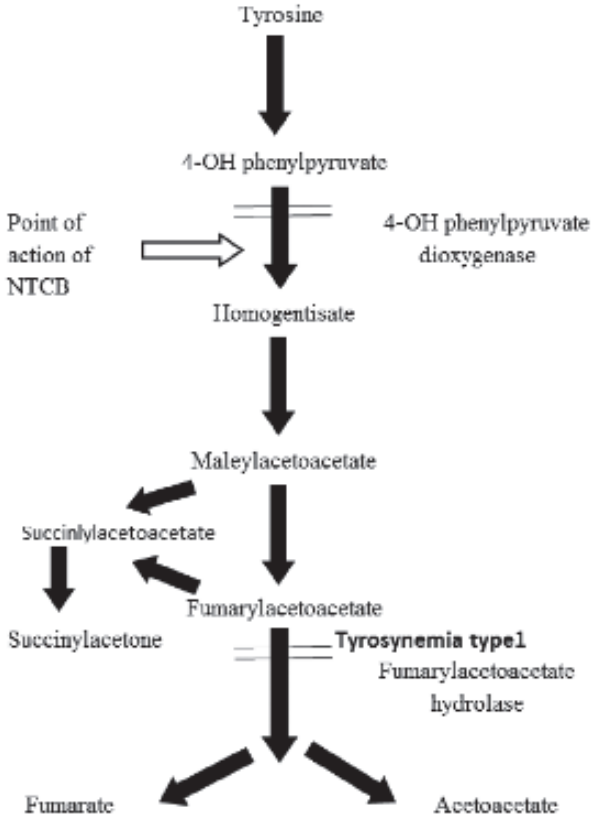

Figure 1. Catabolic pathway of tyrosine
NTCB: 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione

Materials and Methods

Patients

A retrospective study was carried out by twenty nine patients with HTI from twenty three families and who had been followed up by Ege University Faculty of Medicine, Department of Pediatric, Division of Metabolic Diseases and Nutrition Unit, between December 1996 and September 2017. The data of patients including demographic, clinical, biochemical, radiological and mutation analyses were collected from the medical records. Diagnosis of HTI was carried out by detection of elevated SA in urine samples or by molecular genetic analysis or by enzymatic studies. None of the patients were screened at birth for HTI. Except three patients, all of the patients were treated with tyrosine and phenylalanine restricted diet to maintain plasma level below 400 µmol/L as recommended and treated with NTBC at 1-2 mg/kg. Informed constent was obtained.

Statistical Analysis

The quantitative patient characteristics such as age, onset of clinical symptoms were summarized by means and standard deviations (SD). The qualitative characteristics such as hepathomegaly, growth retardation and other organ complications were presented as a frequency distribution.

Results

Demographic Data

We collected data from twenty nine patients from twenty six families diagnosed at Ege University Faculty of Medicine, Department of Pediatric, Division of Metabolic Diseases and Nutrition Units. Aged between one month and one hundred forty months at the time of the diagnosis. Sixteen were males and thirteen were females. There were 29 HTI patients from 23 different families and consanguinity was noted in 14/23 (60.8%) of our patients' families. There were eleven patients in this study who belonged to five sets of families.

Diagnosis and Clinical Presentation

Eight were of acute form, thirteen were of subacute and eight were of chronic form of HTI. None of the patients were screened by expanded newborn screening for HTI.

Two patients were diagnosed with HTI without symptom by selective screening due to affected siblings. First patient (P3S) was diagnosed by selective screening in the newborn period due to an affected sibling history and she was asymptomatic at the diagnosis. We have another asymptomatic patient (P14S) at the time of diagnosis. He was diagnosed with HTI by selective screening while two months old, his brother was also diagnosed with HTI. Mean age onset of clinical symptoms was 3.7±1.6, 9±1.6 and 41±27 months in acute, subacute and chronic HTI patients, respectively. The most common clinical manifestation at the diagnosis was hepatomegaly (96.2%). The other manifestations were respectively rickets, renal tubular dysfunction, growth retardation (Table I).

Laboratory Parameters and Imaging

Hypoglycemia was detected in none of our patients. Anemia was detected in 24 patients and thrombocytopenia was detected in 15 patients at diagnosis (Table II). Renal tubulopathy was detected in 18 patients. Rickets was detected in 20 patients clinically, radiologically or biochemically. Alpha-fetoprotein (AFP) showed a marked increase with a high variability for all ages. Urine SA was detected quantitative in seventeen patients and qualitative in 10 patients. Abdominal ultrasonography showed hepatomegaly in twenty seven patients and liver nodules in twenty patients. Abnormal renal imaging findings include increased renal echogenity; nephromegaly or nephrocalcinosis was seen in sixteen patients. There was no cardiomyopathy in nineteen patients with initial echocardiography.

Table I. Main clinical manifestations at diagnosis

Clinical manifestations	n (%)
Hepatomegaly	26 (96.2)
Rickets	20 (74)
Tubular dysfunction	18 (66.6)
Growth retardation	16 (59.2)
Cirrhosis	13 (48.1)
Bleeding	7 (25.9)
Hepatocellular carsinoma	2 (7.4)
Neurological findings	2 (7.4)

Medication and Diet

Except three patients, all of the patients were treated with tyrosine and phenylalanine restricted diet to maintain plasma level below 400 µmol/L as recommended and treated with NTBC at 1-2 mg/kg. P17 was not treated, because family rejected the treatment. P27 was not treated, because he died within a short time after diagnosis. P29 was not treated, because she came with severe liver failure and liver was transplanted under emergency condition.

Adherence to Therapy

Dietary compliance and NTBC treatment compliance were bad in six patients due to incompatibility of families.

Follow-up

Mean follow-up was 82.2 months (minimum: 1 month-maximum: 203 months). In follow up, regular abdominal

Table II. Biochemical parameters at diagnosis

Parameter	Normal	n	Mean	Range	SD
Plasma					
Hemoglobin (g/dL)	-	29	9.7	7-13.6	1.5
Thrombocyte (/mm^2)	150.000-400.000	29	158.465	29.500-345.000	90.785
AST (IU/L)	0-40	29	169.8	14-1878	347.5
ALT (IU/L)	0-40	29	86.3	12-728	140.4
Total bilirubin (mg/dL)	-	29	2.2	0.4-13.5	2.9
Albumin (g/dL)	3.2-5.4	28	3.9	2.8-4.9	0.6
ALP (IU/L)	60-525	28	1480.6	152-7116	1562.7
AFP (ng /mL)	<7	26	102.012	23-484.000	139.307.4
Tyr (µmol/L)	50-130	21	390.7	96-1430	266.9
Coagulation					
PT (s)	10.4-14	25	22.0	13-36	6.3
aPTT (s)	26-40.8	25	47.5	26-73	12.2
Urine					
SA (quantitative)	<1	17	234	11-992	309.1
SA (qualitative)	Undetectable	10	Increased	-	-

AFP: Alpha-fetoprotein, ALP: Alkaline phosphatase, ALT: Alanine transaminase, aPTT: Activated partial thromboplastin time, AST: Aspartate transaminase, PT: Prothrombin time, SA: Succinylacetone, Tyr: Tyrosine, SD: Standard deviation

Table III. Liver transplntation in hereditary tyrosinemia Type I patients

Patient	HTI subtype	Age at diagnosis (months)	Reason	Age at LT (months)	Type of LT	Result
P3	Subacute	13	Non-compliance with the dietary treatment	17	LDLT	Alive
P5C	Subacute	27	Non-compliance with the dietary treatment	27	LDLT	Alive
P6	Chronic	22	Suspicion of HCC	122	LDLT	Alive
P9	Acute	3	Suspicion of HCC	27	LDLT	Alive
P11	Acute	8	Non-compliance with the dietary treatment	18	LDLT	Alive
P13	Subacute	9	Acute liver failure	10	LDLT	Alive
P15	Chronic	23	Family decision	33	LDLT	Alive
P18	Chronic	71	Suspicion of HCC	121	LDLT	Alive
P19	Acute	7	Family decision	11	LDLT	Alive
P22	Acute	8	Family decision	8	LDLT	Alive
P23	Chronic	92	Suspicion of HCC	92	LDLT	Died after 8 months Hepatorenal syndrom

LT: Liver transplantation, LDLT: Living donor liver transplantation HTI: Hereditary tyrosinemia Type I, HCC: Hepatocellular carcinoma

USG was performed every 6-12 months and AFP levels, plasma amino acids and urine succinylacetone were checked every 3 months. Mayorandan et al.(16) recommended follow-up with ultrasonography (USG) of the liver every 6 months and monitoring of AFP levels every 3-6 months. Eleven patients (P3, P5C, P6, P9, P11, P13, P15, P18, P19, P22, P23) underwent liver transplantation, all living donor transplantations (Table III). After liver transplantation, one patient (P23) died due to hepatorenal syndrome. Porphrya-like crises, in other words neurogenic crises, were detected in four patients (P1S, P2C, P7, P10) by reason of non-compliance with the dietary treatment and NTBC treatment. HCC was detected in five patients (P1S, P2, P8, P12, P17). Two of these patients had HCC at the time of the diagnosis HTI. Two patients who developed HCC during the follow up of HTI were non-compliant with the dietary treatment and NTBC treatment. No adverse effect was seen requiring nitisinone treatment todiscontinue.

Discussion

HTI has a birth incidence approximately 1:125.000 in central Europe. Turkey has a high estimated prevalence of inborn errors of metabolism due to a high rate of consanguineous marriages (17,18). The exact incidence of HTI in Turkey is still unknown. We have 29 HTI patients from 23 different families and consanguinity was noted in 14/23 (60.8%) of our patients' families. This is similar to the other studies of HTI reported from the Turkey (19,20). In Turkey HTI is not a part of national neonatal screening program, so diagnosis still depends on clinical suspicion and laboratory investigations. Two of our patients were diagnosed by selective newborn screening due to an affected sibling and they were asymptomatic at time of the diagnosis. The most common manifestations were respectively hepatomegaly, rickets, renal tubular dysfunction, growth retardation in our patients at the diagnosis. In our study, the first signs were detected after six months of age (19/27) in contrast with report of Couce et al. (21). Also growth retardation was detected at more high ratio (59.2%) in contrast with report of Gokay et al. (19) and Zeybek et al. (20). The mean interval from the first symptom of the diagnosis was 12.2 months. Initial symptoms might have been overlooked by the parents or clinicians especially in the chronic form of HTI. The most remarkable laboratory findings were anemia, elevation of liver enzymes with impairment of the coagulation profile, elevated alkaline phosphatase, AFP and plasma tyrosinelevels. In acute HTI patients, laboratory findings were associated with more severe consistent previous reports (19-21). Urine SA ratio (93.1%) was higher in the report of Gokay et al. (19) and Zeybek et al. (20). Treatment can be started in patients who have response to drug treatment with normalization in serum AFP levels within the first year of therapy. As known, development of HCC is the main risk for patients with the chronic form or who have been treated with NTBC after 2 years of age (22,23). Also, the detection of liver cancer is imperfect and laborious. Two of our patients with HCC were treated with NTBC after 2 years of age. Non-compliance with the NTBC treatment resulted HCC in two patients. Another important point is that permanent interruption of NTBC treatment resulted neurogenic crises in four patients. Önenli Mungan et al. (24) and Schlump et al. (25) reported that interruption of nitisinone treatmentcan cause severe neurological crisis inpatients with HTI. These crises occur in up to 50% of untreated children and these are one of the major causes of mortality (5,26). In follow up, regular abdominal USG was performed every 6-12 months and AFP levels, plasma amino acids and urine succinylacetone were checked every 3 months. Chinsky et al. (27) recommended follow-up with USG or computed tomography or magnetic resonance imaging of the liver yearly and monitoring of AFP levels every 3-6 months. Liver enzymes and coagulation

parameters normalized about 1 week later with diet and nitisinone treatment in all symptomatic patients. Elevated AFP levels of patients who were under medical treatment with NTBC decreased continuously throughout the first year of the treatment. The results of NTBC and diet treatment are consistent with the literature (27). Eleven patients underwent liver transplantation, all living donor transplantations (Table III). Acute liver failure or malignancy are the symptoms of liver transplantation. If the advised medical treatment with nitisinone is not attached to or is not present, the patient is under the risk of acute and chronic complications of HTI. Therefore they may be taken into account for liver transplantation in accordance with the classical criteria determined before the availability of nitisinone. In our study three patients underwent liver transplantation because the families stated that they had a difficulty in adapting medical treatment. Considering this period that can cause a vital hazard patients, liver transplantation request of the families resulted positively. These three patients are still alive.

Study Limitations

Limitations of present study are that the plasma level of nitisinone was not measured and molecular analysis of the patients were not taken into consideration.

Conclusion

NTBC treatment is effective and improves the prognosis of HTI. However, early diagnosis and treatment lead to much better outcome. HCC incidence is low if treatment can be started in newborn period. So, screening for HTI in newborns is very important. Our study showed once again that adherence to the diet and treatment and follow-up schedule of the patients are vital.

Ethics

Informed Consent: Informed constent was obtained.
Peer-review: External and internal peer-reviewed

Authorship Contributions

Surgical and Medical Practices: H.Y., E.E., E.C., S.H.,Concept: H.Y., M.Ç.,Design: H.Y., S.K.U., Data Collection or Processing: H.Y.,Analysis or Interpretation: H.Y.,Literature Search: H.Y.,Writing: H.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Chakrapani A, Gissen P, McKierrnan P. (2016) Disorders of tyrosine metabolism. In: Saudubray JM, van den Berghe G, Walter JH (eds) Inborn metabolic diseases, diagnosis and treatment, 6th edn. Springer, Heidelgerg, pp 265-76.
2. McKiernan PJ, Preece MA, Chakrapani A. Outcome of children with hereditary tyrosinaemia following newborn screening. Arch Dis Child 2015;10:738-41.
3. Angileri F, Bergeron A, Morrow G, et al. Geographical and ethnic distribution of mutations of the fumarylacetoacetate hydrolase gene in hereditary tyrosinemia type 1. JIMD Rep 2015;19:43-58.
4. Morrow G, Angileri F, Tanguay RM.Molecular Aspects of the FAH Mutations Involved in HT1 Disease.Adv Exp Med Biol 2017;959:25-48.
5. van Spronsen FJ, Thomasse Y, Smit GP, et al. Hereditary tyrosinemia type I: a new clinical classification with difference in prognosis on dietary treatment. Hepatology 1994;20:1187-91.
6. Baumann U, Preece MA, Green A, Kelly DA, McKiernan PJ.Hyperinsulinism in tyrosinemia type 1. J Inherit Metab Dis 2005;28:131-5.
7. Arora N, Stumper O, Wright J, Kelly DA, McKiernan PJ.Cardiomyopathy in tyrosinaemia type I is common but usually benign. J Inherit Metab Dis 2006;29:54-7.
8. Mohamed S, Kambal MA, Al Jurayyan NA, et al. Tyrosinemia type 1: a rare and forgotten cause of reversible hypertrophic cardiomyopathy in infancy. BMC Res Notes 2013;6:362.
9. Cassiman D, Zeevaert R, Holme E, Kvittingen EA, Jaeken J. A novel mutation causing mild, atypical fumarylacetoacetase deficiency (Tyrosinemia type I): a case report. Orphanet J Rare Dis2009;4:28.
10. Larochelle J, Alvarez F, Bussières JF, et al. Effect of nitisinone (NTBC) treatment on the clinical course of hepatorenal tyrosinemia in Québec. Mol Genet Metab 2012;107:49-54.
11. Mckiernan PJ. Nitisinone in the treatment of hereditary tyrosinemia type 1. Drugs 2006;66:743-50.
12. Morrow G, Angileri F, Tanguay RM. Molecular Aspects of the FAH Mutations Involved in HT1 Disease. Adv Exp Med Biol 2017;959:25-48.
13. Holme E, Mitchell G. (2014) Tyrosine metabolism. In: Blau N, Duran M, Gibson KM, Dionisi-Vici C (eds) Physician's Guide to the Diagnosis,Treatment, and Follow-Up of Inherited Metabolic Diseases, Springer, Heidelberg, New York, Dordrecht, London, pp 24-31.
14. Lindstedt S, Holme E, Lock EA, Hjalmarson O, Strandvik B.Treatment of hereditary tyrosinaemia type I by inhibition of 4-hydroxyphenylpyruvate dioxygenase. Lancet 1992;340:813-7.
15. Das AM. Clinical utility of nitisinone for the treatment of hereditary tyrosinemia type-1 (HT-1). Appl Clin Genet 2017;10:43-8.
16. Mayorandan S, Meyer U, Gokcay G et al. Cross-sectional study of 168 patients with hepatorenal tyrosinaemia and implications for clinical practice. Orphanet J Rare Dis 2014;9:107.
17. Ozalp I, Coskun T, Tokol S, et al. Inherited metabolic disorders in Turkey. J Inherit Metab Dis 1990;13:732-8.
18. Tuncbilek E, Ozguc M. Application of medical genetics in Turkey. Turk J Pediatr 2007;49:353-9.
19. Gokay S, Ustkoyuncu PS, Kardas F, Kendirci M. The outcome of seven patients with hereditary tyrosinemia type 1. J Pediatr Endocrinol Metab 2016;29:1151-7.
20. Zeybek AC, Kiykim E, Soyucen E, et al. Hereditary tyrosinemia type 1 in Turkey: twenty year singlecenter experience. Pediatr Int 2015;57:281-9.

21. Couce ML, Dalmau J, del Toro M, Pintos-Morell G, Aldamiz-Echevarría L; Spanish Working Group on Tyrosinemia type 1.Tyrosinemia type 1 in Spain: mutational analysis, treatment and long-term outcome. Pediatr Int 2011;53:985-9.
22. Holme E, Lindstedt S. Nontransplant treatment of tyrosinemia. Clin Liver Dis 2000;4:805-14.
23. Van Spronsen FJ, Bijleveld CM, Van Maldegem BT, et al. Hepatocellular carcinoma in hereditary tyrosinemia type I despite 2-(2nitro-4-3-trifluoromethylbenzoyl)-1,3-cyclohexanedione treatment. J Pediatr Gastroenterol Nutr 2005;40:90,3.
24. Önenli Mungan N, Yıldızdaş D, Kör D, et al. Tyrosinemia type 1 and irreversible neurologic crisis after one month discontinuation of nitisone.Metab Brain Dis 2016;31:1181-3.
25. Schlump JU, Perot C, Ketteler K, et al. Severe neurological crisis in a patient with hereditary tyrosinaemia type I after interruption of NTBC treatment. J Inherit Metab Dis 2008;31 Suppl 2:S223-5.
26. Mitchell G, Larochelle J, Lambert M, et al. Neurologic crisis in hereditary tyrosinemia. N Engl J Med 1990;322:432-7
27. Chinsky JM, Singh R, Ficicioglu C, et al. Diagnosis and treatment of tyrosinemia type I: a US and Canadian consensus group review and recommendations. Genet Med 2017;19.

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Original Article

Demographic, Phenotypic and Genotypic Features of Alkaptonuria Patients: A Single Centre Experience

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ABSTRACT

Aim: Alkaptonuria (AKU) is an autosomal recessively inherited disease caused by a deficiency of homogentisate 1,2-dioxygenase. This enzyme converts homogentisic acid (HGA) into maleylacetoacetic acid in the tyrosine degradation pathway. The presence of HGA in urine, ochronosis (bluish-black pigmentation in connective tissues) and arthritis of the spine and the other large joints are the three major features of AKU. Nitisinone and a tyrosine-restricted diet are the treatment options. In this study, we evaluated the demographic and clinical characteristics and also the mutations of our AKU patients.

Materials and Methods: This retrospective single centre study included 36 patients who were diagnosed as AKU between the years of 2002 and 2017 Çukurova University Faculty of Medicine, Department of Pediatrics, Division of Metabolism and Nutrition.

Results: Thirty six AKU patients were included (17 female, 19 male) in our study. The mean age of the patients was 9.3±13.4 years (3 months-54 years). The major complaints were darkening of the urine (100%), ochronosis (11.1%), arthralgia (16.7%) and arthritis (8.1%). Darkening of the urine was firstly recognized at the age of 8.89±16.9 months (1-84 months). Eighteen (86%) patients had homozygous and 3 (14%) patients had compound heterozygous mutations in the *HGD* gene.

Conclusion: AKU was the first inherited metabolic disease defined. The three main features are; darkening of the urine at birth which is followed by ochronosis (blue-dark pigmentation) clinically visible in the ear and alae of the nose and finally a severe ochronotic arthropathy of the spine and large joints at around the age of 50 years. Here we report on the clinical and genetic features of our patients at various ages.

Keywords: Alkaptonuria, ochronosis, homogentisic acid, homogentisate 1,2 dioxygenate, arthritis

Introduction

Alkaptonuria (AKU) was historically used by Archibald Garrod in his lectures in 1908. AKU was one of the first disorders which confirmed the principles of Mendelian recessive inheritance (1). AKU is a rare autosomal recessive disorder with a prevalence of lower than 1:250.000. The deficiency of homogentisate 1,2 dioxygenate (HGD) leads to an accumulation of homogentisic acid (HGA) in plasma and urine which auto-oxidizes in tissues into benzoquinone acetic acid and polymerizes to an ochronotic pigment. Affected individuals excrete HGA in their urine which, when oxidized, causes a characteristic dark color (2). Although black urine and higher HGA levels were seen in the neonatal period, the onset of clinical symptoms is delayed until the second or third decades of life, whereas pigmentation of cartilages develops in childhood (3). As AKU effects different parts of the body, such as the heart, kidney, eye, prostate and gall bladder, it is now considered as a multisystemic disease (4). The underlying mechanism of joint destruction is the binding of HGA to collagen, followed by stiffness of the cartilage matrix and resulting in an additional load on the subchondral bone. Aberrant loading leads to the formation of trabecular excrescences and protrusions. Pigment deposition in the

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heart valves and blood vessels firstly leads to left heart valve calcification, then stenosis or regurgitation and occasionally aortic dilatation (5). Fifty percent of patients with AKU have a history of renal stones, also black prostate stones occur frequently in male patients. The diagnosis of AKU is based on the detection of a significant amount of HGA in a urine sample by gas chromatography or mass spectrometry together with clinical findings. A normal 24-hour urine sample contains 20-30 mg of HGA. The amount of HGA excreted per day is usually between one and eight grams in AKU patients. Management of AKU is based on physical and occupational therapies in order to decrease joint pain and maintain muscle strength and flexibility. Additionally, knee, hip and shoulder may be replaced by prosthesis when needed. A pharmacologic treatment of AKU with low dose nitisinone reduced urinary HGA by up to 95% (6,7). Dietary restriction of phenylalanine and tyrosine is needed in order to reduce elevated levels of tyrosine and hepatic damage as a result of nitisinone treatment, however it is not very practical especially in older patients. High-dose vitamin C also decreases urinary derivatives of HGA (8). In this study, we present the demographic and clinical features of 36 AKU patients who were followed up at our center.

Materials and Methods

Thirty six patients who were diagnosed at Çukurova University Faculty of Medicine, Department of Pediatrics, Division of Metabolism and Nutrition were included in our study. The study was approved by the Ethics in Research Committee of Çukurova University, Faculty of Medicine, Turkey (approval number: 2018/75-56). All patients and/or their legal guardians provided written informed consent. Time of diagnosis and current age, parental consanguinity, clinical findings and mutation analyses were retrospectively investigated. The diagnosis of AKU was based on clinical findings, urinary HGA analyses and mutation analyses.

Statistical Analysis

Data were analysed using "SPSS for Windows 22" software. Descriptive statistics are expressed as mean ± standard deviation or median (minimum-maximum) for discontinuous numeric variables and categorical variables are expressed as case number and percentage.

Results

The mean age of diagnosis was 9.3±13.4 years (3 months-54 years) although the mean age of the first complaint was 8.89±16.9 months (1-84 months). There were 17 females (52.8%) and 19 males (47.2%). The ratio of consanguinity between the parents was 83.3%. Nineteen patients (52.8%) had a family history of AKU. A black staining of the diaper in the newborn period was the first and main complaint of 17 patients (47.2%). Other complaints were darkening of urine color (100%), ochronosis 4 (11.1%) (Figure 1), arthralgia 6 (16.7%), and arthritis 3 (8.1%). The youngest patient who was examined at our clinic with bilateral knee pain was 12 years old. Three patients had celiac disease, congenital hypothyroidism and Gilbert syndrome in addition to AKU. Two siblings from the same family were admitted to the physical rehabilitation department with hip pain when they were over 50 years of age. Immediately after they were diagnosed with osteoarthritis, a bilateral total hip replacement was performed (Figure 2). The clinical symptoms were similar in these patients, with darkening of the urine in childhood, skin and scleral discoloring in youth and ochronotic arthropathy (Figure 3) in middle age. The main complaint of three siblings from another family was blue colored macular pigmentation. Compared to other patients this symptom was only seen in this family. These patients had the p.R58fs mutation in the *HGD* gene. Twenty one patients were screened for mutations in the *HGD* gene. A total of 8 different mutations were detected. Eighteen (86%) patients had homozygous and 3 (14%) patients had compound heterozygous mutations in the *HGD* gene. To the best of our knowledge, two mutations have never been reported previously: one of them was a frame shift mutation and the other was a mutation of unknown clinical significance. Fourteen of them were missense mutations. The most frequent mutation was p.R58fs/c.175 del which

Figure 1. Auricular cartilages with ochronosis of family number 4

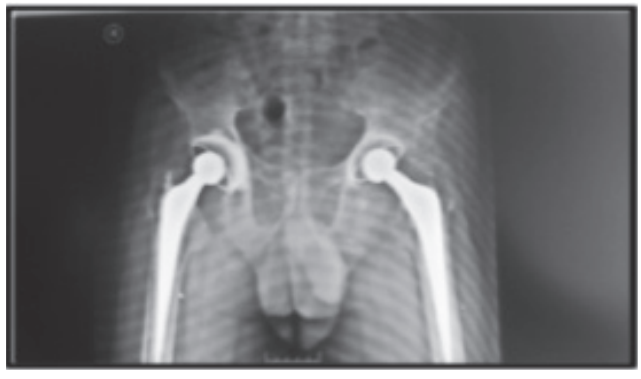

Figure 2. Total hip arthroplasty of family number 4

was detected in 9 alleles. The second most frequent mutation was p.R225H/c.674G>A. We found this mutation in 5 alleles and two of them were from the same family. Three patients had compound heterozygous mutations: p.R58fs [(c.175delA)/p.R225H (c.674G>A)], two of them being from the same family (Table I). A mild protein restricted diet and vitamin C were administered to those patients who had joint pain, arthritis or ochronosis.

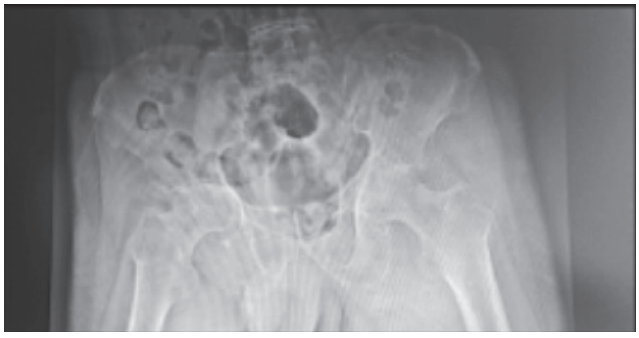

Figure 3. Ochronotic arthropathy of family number 4

Discussion

AKU is characterized by HGA accumulation in tissues. The disease occurs as a result of a defect in the *HGD* gene. HGA is the main product of tyrosine and phenylalanine. The *HGD* gene breaks down HGA. Ochronosis is a diagnostic feature of AKU and is seen in nearly 50% of AKU patients (9). The main accumulation site of blue-brownish pigmentation is observed in cartilage rich tissues such as the nose and ear and is rarely seen at early ages. The accumulation of blue-brownish pigment in conjunctiva, cornea and sweat glands causes staining on clothes (10). The mean age of diagnosis of our patients was 9.3±13.4 years. The ratio of consanguinity between parents was 83.3%. AKU patients generally present with either dark urine or early onset of arthritis. In our study, the diagnosis was based upon complaints of dark urine in all cases, joint pain in 16.7% and ochronosis in 11.1% of cases. AKU generally affects large joints such as the shoulder, hip and knee and arthritis develops in later stages. Severe pain, stiffness and ochronosis are frequently observed symptoms.

Table I. Mutation analysis results of 21 patients

	Current age/gender	Protein	Nucleotide	Type	Clinical findings				
					Black urine	Ochronosis	Arthralgia	Arthritis	Macular pigmentation
1	4y/M	p.R58fs/p.R225H	c.175delA/c.674G>A	Frameshift/missense (CH)	+	-	-	-	-
	9y5m/M	p.R58fs/p.R225H	c.175delA/c.674G>A	Frameshift/missense (CH)	+	-	-	-	
2	14y4m/M	p.R58fs	c.175delA	Frameshift (H)	+	-	-	-	+
	15y8m/F	p.R58fs	c.175delA	Frameshift (H)	+	+	+	-	+
	6y5m/F	p.R58fs	c.175delA	Frameshift (H)	+	-	-	-	+
3	7y/M	p.R225H	c.674G>A	Missense (H)	+	-	-	-	-
	2y/M	p.R225H	c.674G>A	Missense (H)	+	-	-	-	-
4	54y8m/M	p.L25P	c.74T>C	Missense (H)	+	+	+	+	-
	50y2m/F	p.L25P	c.74T>C	Missense (H)	+	+	+	+	-
5	12y9m/M	p.R225H	c.674G>A	Missense (H)	+	-	-	-	
6	2y1m/M	p.G270R	c.808G>A	Missense (H)	+	-	-	-	-
7	6y1m/M	p.R336K	c.1007G>A	Missense (H)	+	-	-	-	-
8	10y9m/M	p.F227L	c.679T>C	Missense (H)	+	-	-	-	-
9	2y11m/F	p.R225H	c.674G>A	Missense (H)	+	-	-	-	-
10	14y2m/F		c.1189_41_1249del102bp	Frameshift (H)	+	-	+	-	-
11	7y9m/F	p.R58fs/p.R225H	c.175delA/c.674G>A	Frameshift/missense (CH)	+	-	-	-	-
12	10y5m/F	p.R58fs	c.175delA	Frameshift (H)	+	-	-	-	-
13	7y2m/F	p.M368V	c.1102A>G	Missense (H)	+	-	-	-	-
14	12y9m/M	p.R225H	c.674G>A	Missense (H)	+	-	-	-	-
15	6y1m/F	p.R58fs	c.175delA	Frameshift (H)	+	-	-	-	-
16	7y7m/F	p.R58fs	c.175delA	Frameshift (H)	+	-	-	-	-

F: Female, M: Male, CH: Compound heterozygous, H: Homozygous

Two of our elderly patients were referred to us with pain, stiffness and ochronosis of large joints. Blue or mottled macules appear on fingers, ears, nose, genital regions, axillae and the buccal mucosa. Palmoplantar pigmentations may also occur. The sweat glands are rich from ochronotic pigment granules (11). Our three patients from the same family had bluish macules on their forehead, neck, hands and back. Uyguner et al. (12) reported that p.R58fs and p.R225H are the most common *HGD* mutations in Turkey, which provides a novel insight into the origins and migration of common European AKU mutations. With the analysis of seven unrelated families and 14 affected individuals from different regions of Turkey, patients from three families were homozygous for the p.R58fs mutation; three other families were homozygous for the p.R225H mutation; and one family was homozygous for the p.G270R mutation. We detected the p.G270R mutation in only one patient in our study. The previously reported haplotypes revealed that p.R225H is a recurrent mutation in Turkey. These analyses showed that p.R58fs is an old AKU mutation that probably originated from central Asia and spread throughout Europe and Anatolia (12). In our study, the most common mutation was p.R58fs and the second was p.R225H. To date, 149 different *HGD* variants have been identified. All variants are summarized in the HGD mutation database (13-15). p.R58fs is one of the first identified AKU mutations (16-19). At the same time, there are variants rather specific to some countries or regions. So far, 950 AKU patients have been reported in 61 countries worldwide (AKU Society, www.akusociety.org). The highest number of AKU patients was reported in Slovakia (20). Slovakia and the Dominican Republic exhibit a prevalence of AKU up to 1:19 000 (21,22). Recently, a high number of AKU cases were also found in Jordan (23) and India (18). Nemethova et al. (24) reported 99 AKU patients with 12 novel mutations from Italy. In our study, 8 different AKU mutations with two new mutations were identified. The most frequently presented forms were missense mutations, followed by frameshift mutations. p.M368V was the most prevalent AKU mutation in Europe (24). This mutation was detected in only one patient in our study. Similarly, other AKU mutations reported from different countries like p.IVS1-1GrA, p.V300G and p.P230S were also rare in our patient group. We observed p.R336K mutation in one patient with unknown clinical significance. We also found p.F227L mutation in one patient that had not been defined previously. We evaluated its possible pathogenicity using different bioinformatic prediction tools: PolyPhen-2, SIFT, MutationTaster and therefore believe p.F227L to be possibly damaging. There is no definite therapeutic protocol or no effective treatment for this disease. However, ascorbic acid combined with a therapy of nitisinone with a dietary restriction of phenylalanine and tyrosine are the recommended modalities of treatment. Different clinical trials analyzed the effectiveness of a low-protein diet and ascorbic acid treatments. Vitamin C at an experimental dose of 100 mg/day has been shown to reduce ochronotic pigment accumulation and HGA urinary excretion. On the other hand, it was found to increase HGA production, contributing to the formation of renal oxalate stones. A mild protein restricted diet and vitamin C treatment were begun in symptomatic patients who had arthralgia, arthritis or ochronosis in our study. Nitisinone inhibits the enzyme that produces HGA but, at present, it is still under trial and its use is limited to adult alkaptonuric patients (25,26). Additional studies will need to examine the clinical outcomes of the various treatment strategies.

Study Limitations

This study has some limitations. We did not perform mutation analysis on all the patients and the sample size was small.

Conclusion

Here, we reported our AKU patients with various signs and symptoms at different ages. Additionally, we documented clinical and genetic features of our patients. AKU is a rarely seen metabolic disorder causing cosmetic problems in childhood, serious arthropathy and cardiac valve calcifications in adulthood. Life quality of patients is affected.

Ethics

Ethics Committee Approval: The study was approved by the Ethics in Research Committee of Çukurova University Faculty of Medicine, Adana, Turkey (approval number: 2018/75-56).

Informed Consent: Consent form was filled out by all participants.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.Ş.Y., N.Ö.M., Concept: T.S., S.B., Design: S.K., D.K., Data Collection and Processing: N.Ö.M., S.K., Analysis and Interpretation: N.Ö.M., F.D.B., Literature Search: F.D.B., S.K., Writing: S.K.

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References

1. Garrod AE. The incidence of alkaptonuria: a study in chemical individuality. 1902 [classical article]. Yale J Biol Med 2002;75:221-31.
2. Mannoni A, Selvi E, Lorenzini S, et al. Alkaptonuria, ochronosis, and ochronotic arthropathy. Semin Arthritis Rheum 2004;33:239-48.
3. Arnoux JB, Le Quan Sang KH, et al. Old treatments for new insights and strategies: proposed management in adults and children with alkaptonuria. J Inherit Metab Dis 2015;38:791-6.
4. Helliwell TR, Gallagher JA, Ranganath L. Alkaptonuria--a review of surgical and autopsy pathology. Histopathology 2008;53:503-12.

5. Hannoush H, Introne WJ, Chen MY, et al. Aortic stenosis and vascular calcifications in alkaptonuria. Mol Genet Metab 2012;105:198-202.
6. Anikster Y, Nyhan WL, Gahl WA. NTBC and alkaptonuria. Am J Hum Genet 1998;63:920-1.
7. Suwannarat P, O'Brien K, Perry MB, et al. Use of nitisinone in patients with alkaptonuria. Metabolism 2005;54:719-28.
8. Tinti L, Spreafico A, Braconi D, et al. Evaluation of antioxidant drugs for the treatment of ochronotic alkaptonuria in an in vitro human cell model. J Cell Physiol 2010;225:84-91.
9. Capkin E, Karkucak M, Yayli S, Serdaroğlu M, Tosun M. Ochronosis in differential diagnosis of patients with chronic backache: a review of the literature. Rheumatol Int 2007;28:61-4. Epub 2007 Jun 13
10. Thomas M, Jebaraj JI, Thomas M, George R. Acral pigmentation in alkaptonuria resembling degenerative collagenous plaques of the hands: a report of five cases. J Am Acad Dermatol 2011;65:e45-6.
11. Odom RB JW, Berger TG. Diseases of the Skin. 9th ed ed. Error in metabolism. 2000, Philadelphia: WB Saunders Company.
12. Uyguner O, Goicoechea de Jorge E, Cefle A, et al. Molecular analyses of the HGO gene mutations in Turkish alkaptonuria patients suggest that the R58fs mutation originated from central Asia and was spread throughout Europe and Anatolia by human migrations. J Inherit Metab Dis 2003;26:17-23.
13. http://hgddatabase.cvtisr.sk/.
14. Yang YJ, Guo JH, Chen WJ, et al. First report of HGD mutations in a Chinese with alkaptonuria. Gene 2013;518:467-9.
15. Zatkova A, Sedlackova T, Radvansky J, et al. Identification of 11 Novel Homogentisate 1,2 Dioxygenase Variants in Alkaptonuria Patients and Establishment of a Novel LOVD-Based HGD Mutation Database. JIMD Rep 2012;4:55-65.
16. Zatkova A. An update on molecular genetics of Alkaptonuria (AKU). J Inherit Metab Dis 2011;34:1127-36.
17. Zatkova A, de Bernabe DB, Polakova H, et al. High frequency of alkaptonuria in Slovakia: evidence for the appearance of multiple mutations in HGO involving different mutational hot spots. Am J Hum Genet 2000;67:1333-9. Epub 2000 Oct 2
18. Sakthivel S, Zatkova A, Nemethova M, et al. Mutation screening of the HGD gene identifies a novel alkaptonuria mutation with significant founder effect and high prevalence. Ann Hum Genet 2014;78:155-64.
19. Goicoechea De Jorge E, Lorda I, Gallardo ME, et al. Alkaptonuria in the Dominican Republic: identification of the founder AKU mutation and further evidence of mutation hot spots in the HGO gene. J Med Genet 2002;39:E40.
20. Srsen S, Müller CR, Fregin A, Srsnova K. Alkaptonuria in Slovakia: thirty-two years of research on phenotype and genotype. Mol Genet Metab 2002;75:353-9.
21. Milch RA. Studies of Alcaptonuria: Inheritance of 47 Cases in Eight Highly Inter-related Dominican Kindreds. Am J Hum Genet 1960;12:76-85.
22. Srsen S, Varga F. Screening for alkaptonuria in the newborn in Slovakia. Lancet 1978;2:576.
23. Al-Sbou M, Mwafi N. Nine cases of Alkaptonuria in one family in southern Jordan. Rheumatol Int 2012;32:621-5.
24. Nemethova M, Radvanszky J, Kadasi L, et al. Twelve novel HGD gene variants identified in 99 alkaptonuria patients: focus on 'black bone disease' in Italy. Eur J Hum Genet 2016;24:66-72.
25. Mayatepek E, Kallas K, Anninos A, Müller E. Effects of ascorbic acid and low-protein diet in alkaptonuria. Eur J Pediatr 1998;157:867-8.
26. de Haas V, Carbasius Weber EC, de Klerk JB, et al. The success of dietary protein restriction in alkaptonuria patients is age-dependent. J Inherit Metab Dis 1998;21:791-8.

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Original Article

An Evalution of the Demographic and Clinical Characterictics of Patients with GM2 Gangliosidosis

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ABSTRACT

Aim: The purpose of our study is to submit the demographic, phenotypic and age at diagnosis characteristics of children with GM2 gangliosidosis.

Materials and Methods: Patients with GM2 gangliosidosis who were referred to Ege University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Nutrition and Metabolism between January 2004 and December 2016, were included in this study. Diagnosis was confirmed by determining the level of serum β-hexosaminidase activity and genetic mutation analysis. The demographic and clinical features are reported for 8 patients with Tay-Sachs disease (TSD) and 6 with Sandhoff disease.

Results: The mean age at diagnosis was 18.2 months (range 4-48 months) and 14.5 months (range 8-36 months) for patients with TSD or Sandhoff disease respectively. The initial and main complaint in 100% of the patients were neurological disorders, such as developmental delay, developmental regression or both; seizures and macrocephaly. None of the patients exhibited evidence of organomegaly. Cranial magnetic resonance imaging results were normal in 36% of the cases, 55% of the cases had bilateral thalami involvement presenting as T2 hyperintensity especially at the posterior thalami and 9% of cases had myelination delay.

Conclusion: GM2 gangliosidosis disease should be considered for children with developmental regression and/or delay. To prevent a delay in diagnosis, β-hexosaminidase activity in serum and genetic mutation analysis should be undertaken in suspected cases. Curative gene therapy may be available in the future.

Keywords: GM2 gangliosidosis, hexoaminidase, Tay-Sachs disease, Sandhoff disease

Introduction

GM2 gangliosidoses including Tay-Sachs disease [TSD, Online Mendelian Inheritance in Man (OMIM) 272800], Sandhoff disease (SD, OMIM 268800) and GM2 activator protein deficiency (GM2; OMIM 272750) are rare lysosomal storage disorders of the sphingolipid metabolism due to an autosomal recessive inheritance. These disorders are characterized by a disrupted lysosomal defect of glycosphingolipid, accumulating in the organelle with the respective glycoconjugates. GM2 gangliosides occur due to the enzymatic presentation of impaired activity of hydrolase b-hexosaminidase or rarely from defects in GM2 activator protein. The incidence of TSD and SD is common in some populations, Ashkenazi Jewish or individuals of French Canadian decent (1-3).

A hereditary defect in the hexosaminidase A-subunit gene (*HEXA*, chromosome 15) or hexosaminidase B-subunit gene (*HEXB*, chromosome 5) results in the absence of (HEX, E.C.3.2.1.52) isoenzymes.

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TSD and its variants arise from mutations in the *HEXA* gene and are associated with deficient HEXA activity but normal HEXB activity. SD and its variants are caused by mutations in the *HEXB* gene and are associated with a deficiency of both HEXA and HEXB activity. GM2 gangliosidosis, AB variant, is a rare form of GM2 gangliosidosis resulting from a lack of GM2 activator protein, it is associated with autosomal recessive mutations in GM2A. Defects in any of these three genes result in an excessive accumulation of GM2 and related glycolipids, mostly in lysosomes in neural cells, and form a rare neurodegenerative disorder (4-8).

Some people with SD are clinically indistinguishable except for visceral and skeletal indications (9,10). These disorders may occur at almost any age with neurodegeneration which progresses at a variable rate. Patients with the classical onset of the disease suffer an accumulation in the retinal ganglion cells leading to the "cherry-red spot"; these children succumb to prompt neurodegeneration and pass away in early childhood. Less frequently, juvenile-onset forms present with progressive cerebellar dysfunction, dementia and spasticity. Rarely, adult-onset variants occur; but the symptoms of disease present in childhood (11-15). No curative therapy is available for GM2 gangliosidosis. Seizures generally respond to standard treatment.

Fourteen patients with GM2 gangliosidoses, referred to Ege University Faculty of Medicine, Department of Pediatrics, Division of Inborn Error of Metabolism, were included in our study. We evaluated age, gender, medical background, developmental state, clinical features and the neuroimaging findings of these individuals.

Materials and Methods

The medical records of 14 children with GM2 gangliosidosis who were referred to Ege University Faculty of Medicine, Department of Paediatrics, Division of Inborn Error of Metabolism between January 2004 and December 2016 were retrospectively evaluated.

The hospital records of the patients included in our study were reviewed. We assessed age, gender, past medical background, developmental state and the neuroimaging findings of these individuals. Their clinical manifestations and neuroimaging findings were the factors leading to their diagnosis which was confirmed by analysis of β-hexosaminidase activity in their serum.

Descriptive methods were used to analyze data and statistical testing was not performed. Written consent was obtained from all parents who participated in this survey.

Statistical Analysis

The quantitative analysis of patients is presented as means. The qualitative characteristics are summarized as a frequency distribution.

Results

In our study, 14 individuals (3 female, 11 male) diagnosed with GM2 gangliosidosis [8 (57%) with TSD, 6 (43%) with SD] were included. Consanguinity was reported in 10 (71.4%) individuals. Four patients (28.5%) had a positive family history. The first symptoms of these GM2 diseases were noticed at the mean age of 8.6 months (range 4-14 months) for patients with TSD and 8.5 months (range 4-13 months) for patients with SD.

With respect to their developmental evaluation, 71% of patients demonstrated developmental regression. The mean age of developmental regression was 12.3 months. Developmental regression occurred at a mean age of 9 months (range 4-16 months) and 17.25 months (range 8-22 months) for patients with TSD and SD respectively.

Neurologic features present singularly or with other properties, such as developmental delay or regression in 11 of 14 (79%), strabismus or perceived visual degradation in two of 14 (14%), hyperacusis with extreme startle in 2 of 14 (14 %) and seizures in 9 of 14 (64%) [mean age of 14 months (range 4-28 months)] children with GM2 gangliosidosis.

None of the children with GM2 gangliosidosis disease achieved independent walking. Thirteen (93%) patients had combined axial hypotonia and limb spasticity. Retinal storage leading to a visible cherry-red spot was reported in 12 of 14 (86%) individuals.

Two of the patients had a distinct facial appearance, namely a protuberant forehead, a depressed nasal bridge and hypertelorism. Weight in 3 of 14 (21%) patients less than the 3rd percentile and height in 4 of 14 (29%) patients less than the 3rd percentile.

Three patients showed macrocephaly. None of the patients exhibited any evidence of organomegaly. One patient had rocker bottom foot, a valgus and equinus deformity of the foot (Figure 1). In laboratory data, three patients had increased levels of aspartate aminotransferase and alanine aminotransferase.

The neuroimaging data revealed that 36% of the patients had normal neuroimaging results; 55% of patients had bilateral thalami presenting as T2 hyperintensity of the

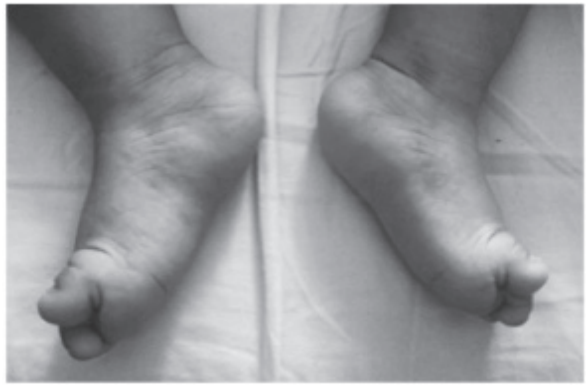

Figure 1. Rocker bottom foot, valgus and equinus deformity of the foot is demonstrated

posterior thalami; 9% of the patients showed myelination delay.

The mean age at diagnosis, determined by β-hexosaminidase activities, was 14.5 months (range 8-36 months) and 18.2 months (range 4-48 months) for individuals with TSD and SD respectively. A delay of 4.6 months (range 1-13 months) and 10.6 months (range 1-40 months) from symptom onset to diagnosis was seen in TSD and SD respectively (overall average of 8 months).

The β-hexosaminidase A enzyme activity, the total β-hexosaminidase enzyme activity and mutation analysis detected in our Tay-Sachs and Sandhoff patients are shown in Tables I and II respectively.

Death was recorded in 10 of the 14 patients (71.5%) and occurred at a mean age of 29.4 months (range 19-45 months). The cause of death was pneumonia and/or respiratory failure in 8 (57%) children. For the remaining 6 patients (43%) in the study, the cause of death is unknown.

Discussion

GM2 gangliosidoses are caused by a lysosomal storage of ganglioside GM2 and related gangliosphingolipids in neurons and glial cells. TSD and SD are a result of lysosomal β-HEXA deficiencies caused by gene mutations in *HEXA and HEXB*, respectively encoding the α- and β-subunits of HEXA (αβ heterodimer) and are associated with an excessive accumulation of GM2 in the brain, which leads to neurological symptoms. The clinical phenotype of the classic infantile form of GM2 gangliosidosis is characterized by normal early development followed by developmental regression, progressive weakness, exaggerated startle, vision loss with cherry-red spots and seizures (8).

In this study, although the number of male patients is higher than female patients, there were no significant gender related differences.

The mean age at diagnosis was 14.5 months (range 8-36 months) and 18.2 months (range 4-48 months) for patients with TSD and SD respectively. Similar to our study, Gort et al. (16) showed that the age at diagnosis range for TSD patients was between 7-36 months and the age at diagnosis range for SD patients was between 7-21 months.

With respect to their developmental evaluation, 71% of individuals showed developmental regression. The mean age of developmental regression was 12.3 months. According to an Iranian paediatric case series based on 18 patients with GM2 gangiosidosis, 66% of patients presented developmental regression and the mean age of developmental regression was 15 months, which is similar to our findings (17).

Multiple seizures types were noted, for example generalized convulsive seizures and myoclonic seizures. Gort et al. (16) mentioned that seizures occurred in individuals with infantile-onset disease at an average age of 15.1 months (range 4-30 months), which is similar to our study.

Table I. Genotype and total hexosaminidase levels in Sandhoff disease patients

Patient	Allel 1	Allel 2	Total HEX level in serum
1	-	-	512 nmol/h/mg (4500-17000)
2	-	-	9.14 nmol/h/mg protein (1097-1341)
3	-	-	15 nmol/h/mg protein (600-2675)
4	c.995>C (p.F332S)	c.995>C (p.F332S)	48.09 nmol/h/mg protein (1223±273)
5	c.156_170del15bp	IVS11+5G>A	61 nmol/h/mg protein (1223±273)
6	-	-	7.5 nmol/mL/h (104-321)

HEX: Hexosaminidase

Table II. Genotype and hexosaminidase A levels in Tay-Sachs disease patients

Patient	Allel 1	Allel 2	HEXA level in serum
1	c.1096_1107del12bp (p.366_369delYGKG)	c.1096_1107del12bp (p.366_369delYGKG)	4.79 nmol/h/mg protein (116±39)
2	-	-	7.3 umol/L/h (50-250)
3	-	-	2.23 nmol/h/mg protein (116±39)
4	c.902 T>G (M301R)	c.902 T>G (M301R)	2.8 umol/L/h (50-200)
5	c.1099_1110del12bp (p.336_369delYGKG)	c.1099_1110del12bp (p.336_369delYGKG)	2.92 nmol/h/mg protein (116±39)
6	-	-	1.5 nmol/h/mg protein (116±39)
7	c.798G>C (p.W266C)	c.798G>C (p.W266C)	1.18 nmol/h/mg protein (116±39)
8	-	-	1.3 nmol/mL/h (7-70)

HEXA: Hexosaminidase A

Typical fundal variations are rare in the juvenile onset variant of the disease, although loss of vision due to optic atrophy and retinitis pigmentosa may occur (12-15). Kokot et al. (18) made appropriate diagnoses of Sandoff and TSD in their individuals based on a prompt eye fundus inspection and the determination of the cherry-red spots in the central area. Cherry-red spots were detected in 88% of the Iranian paediatric case series, which is similar to our cases (17).

Organomegaly was not determined in any of the patients in our study. Similarly, Barness et al. (19) did not mention organomegaly for individuals diagnosed with SD.

In our study, 36% of patients had normal neuroimaging results; 55% of patients had bilateral thalami presenting as T2 hyperintensity of the posterior thalami; 9% of patients exhibited myelination delay. Yun and Lee (20) mentioned low signal intensity in the thalamus and high signal intensity in the white matter of brain in T2 weighted magnetic resonance imaging.

The large number of the published reports, which are based on single case reports or a small number of cases or alternatively retrospective cross-sectional studies (a clinical data report obtained over a period of time) lack information on the patients' mutations. The correlation between the disease progression and the phenotype of the genotype is speculative since the number of patients is generally low with these diseases. The genetic analysis of one patient revealed c.798G>C in the *HEXA* gene. This mutation is a novel mutation which causes the disease according to the modelling program. Segregation analysis from parents is planned to be conducted.

In this study, we reported that the mean age at death was 29.4 months (range 19-45 months), pneumonia and/or respiratory insufficiency was the cause of death for 57% of the patients, with reason of death unspecified in the remaining 43% of the children. Smith et al. (21) reported that the mean age at death was 36.3 months (18 months-6years 9 months) and pneumonia/respiratory failure was the cause of death for 58% of children with GM2 gangliosidoses.

Study Limitations

The limitation of the current study is that molecular analysis of all patients cannot be performed.

Conclusion

GM2 gangliodiosis are rare, but have severe presentation. In order to diagnose earlier, β-hexosaminidase activity assay should be undertaken when GM2 gangliodiosis are suspected. Currently, curative treatment is not available, however, gene therapy may be the hope that provides clinical improvement. Fundus examination should be evaluated in infants with developmental delay and/or developmental retardation and GM2 gangliosidoisis should be considered in differential diagnosis of patients with cherry-red spot.

Ethics

Informed Consent: Informed consent was obtained.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.E., E.C., H.Y., C.E., E.S., Concept: S.K.U., Design: M.Ç., Data Collection or Processing: E.C., Analysis and Interpretation: H.Y., Literature Search: E.E., Writing: H.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Meikle PJ, Hopwood JJ, Clague AE, et al. Prevalence of lysosomal storage disorders. JAMA 1999;281:249-54.
2. Andermann E, Scriver CR, Wolfe LS, et al. Genetic variants of Tay-Sachs disease: Tay-Sachs disease and Sandhoff's disease in French Canadians, juvenile Tay-Sachs disease in Lebanese Canadians, and a Tay-Sachs screening program in the French- Canadian population. Prog Clin Biol Res 1977;18:161-88.
3. Myrianthopoulos N. Some Epidemiological and Genetic Aspects of Tay Sachs Disease. (Academic Press, New York, 1962.).
4. Sandhoff K. The GM2-gangliosidoses and the elucidation of the beta-hexosaminidase system. Adv Genet 2001;44:67-91.
5. Gravel RA, Triggs-Raine BL, Mahuran DJ. Biochemistry and genetics of Tay-Sachs disease. Can J Neurol Sci 1991;18:419-23.
6. Martino S, Cavalieri C, Emiliani C, et al. Restoration of theGM2 ganglioside metabolism in bone marrow derived stromal cells from Tay-Sachs disease animal model. Neurochem Res 2002;27:793-800.
7. Martino S, Emiliani C, Tancini B, et al. Absence of metabolic cross-correction in Tay-Sachs cells:implications for gene therapy. J Biol Chem 2002;277:20177-84.
8. Gravel R, Kaback M, Proia R, et al. The GM2 gangliosidosis. In: Scriver CR, Beaudet A, Sly WS, Valle D, eds. The Metabolic and Molecular Basis of Inherited Disease. 8. New York: McGraw-Hill 2001;3827-76.
9. Sandhoff K, Andreae U, Jatzkewitz H. Deficient hexozaminidase activity in an exceptional case of Tay–Sachs disease with additional storage of kidney globoside in visceral organs. Life Sci 1968;7:283-8.
10. Krivit W, Desnick RJ, Lee J, et al. Generalized accumulation of neutral glycosphingolipids with GM2 ganglioside accumulation in the brain. Sandhoff disease [variant of Tay–Sachs disease]. Am J Med 1972;52:763-70.
11. JohnsonWG. The clinical spectrum of hexosaminidase deficiency disease. Neurology 1981;31:1453-6.
12. Maegawa G, Stockley T, Tropak M, et al. The natural history of juvenile or subacute GM2 gangliosidosis: 21 new cases and literature review of 134 previously reported. Pediatrics 2006;118:e1550-62.
13. Neudorfer O, Pastores GM, Zeng BJ, Gianutsos J, Zaroff CM, Kolodney EH. Late-onset Tay–Sachs disease: phenotypic characterization and genotypic correlations in 21 affected patients. Genet Med 2005;7:119-23.

14. Federico A, Palmeri S, Malandrini A, Fabrizi G, Mondelli M, Guazzi GC. The clinical aspects of adult hexosaminidase deficiencies. Dev Neurosci 1991;13:280-7.
15. Gravel RA, Kaback MM, Proia RL, Sandhoff K, Suzuki K, Suzuki K. The GM2 gangliosidoses. In: Valle D, Beaudet AL, Vogelstein B, Kinsler KW, Antonarakis SE, Ballabio A, editors. The Online Metabolic & Molecular Bases of Inherited Disease. Scriver's OMMBID. New York: McGraw-Hill, 2010: http://dx.doi.org/10.1036/ommbid.184.
16. Gort L, de Olano N, Macías-Vidal J, Coll MA; Spanish GM2 Working Group. GM2 gangliosidoses in Spain: analysis of the HEXA and HEXB genes in 34 Tay-Sachs and 14 Sandhoff patients. Gene 2012 Sep 10;506:25-30. Epub 2012 Jul 10
17. Karimzadeh P, Jafari N, Nejad Biglari H, et al. GM2-gangliosidosis (Sandhoff and Tay Sachs disease): Diagnosis and neuroimaging findings (an Iranian pediatric case series). Iran J Child Neurol 2014;8:55-60.
18. Kokot W, Raczyńska K, Krajka-Lauer J, Iwaszkiewicz-Bilikiewicz B, Wierzba J. Sandhoff's and Tay-Sachs disease–based on our own cases. Klin Oczna 2004;106(3 Suppl):534-6.
19. Barness LA, Henry K, Kling P, et al. A 7-year old white-male boy withprogressive neurological deterioration. Am J Med Genet 1991;40:271-9.
20. Yun YM, Lee SN. A case refort of Sandhoff disease. Korean J Ophthalmol 2005;19:68-72.
21. Smith NJ, Winstone AM, Stellitano L, Cox TM, Verity CM. GM2 gangliosidosis in a UK study of children with progressive neurodegeneration: 73 cases reviewed. Dev Med Child Neurol 2012;54:176-82.

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Original Article

False Positive Diagnosis of Lysosomal Storage Disease Based on Dried Blood Spot Sample; Leucocyte Number of a Challenging Factor

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ABSTRACT

Aim: Recently dried blood spot (DBS) samples have been recommended as a screening test for Lysosomal Storage diseases. Although DBS samples have many advantages including non-invasiveness, cost and transportation, usage of these samples is limited by its high false positive rate. We aimed to investigate any possible effect of the leucocyte number on enzyme activity in dried blood samples in a retrospective study.

Materials and Methods: Data was collected from subjects (n=263) for whom hematological parameters were available in the database of Ege University Hospital. The lysosomal enzyme activity results (alpha glycosidase, glycocerebrosidase, alpha galactosidase, sphingomyelinase and galactocerebrosidase) were re-evaluated with regard to the leucocyte number. Enzyme activities were measured using fluorometric and liquid chromatography-tandem mass spectrometry methods.

Results: All enzyme activities closely correlated with the total number of leucocyte, since leucocytes are the main source of lysosomal enzymes. Glycocerebrosidase and galactocerebrosidase presented a positive correlation with the number of neutrophils and sphingomyelinase showed a positive correlation with the number of lymphocytes. When we recalculated the lysosomal enzyme activities with regard to the leucocyte number, the false positive rates for glycocerebrosidase, sphingomyelinase and alpha galactosidase decreased from 20%, 10.5% and 10.8% to 4.5%, 4.4% and 4.2%, respectively.

Conclusions: Our data indicated that the enzyme activity in dried blood samples including low leucocyte number might be found lower than reference intervals resulting in false positive diagnosis. We concluded that the calculation of enzyme activity with regard to the number of leucocytes might produce more reliable results and might be helpful in decreasing the false positive rate.

Keywords: Dried blood spot, leucocyte number, lysosomal storage disease

Introduction

Lysosomal Storage diseases (LSD), which are related to a deficiency of specific lysosomal hydrolases, resulted in clinical aspects due to an accumulation of substrates in different tissues. It is critically important to diagnose patients in the early stages of this disease since enzyme replacement therapy might prevent complications and also increase life expectancy. Although the determination of enzyme activities in leucocyte and/or fibroblast samples are recommended as the gold standard, these samples are not preferred for screening due to their disadvantages; namely; sampling is invasive, the method is expensive, fast transportation is essential etc (1-3). Since dried blood spot (DBS) is non-invasive, low-cost, easily transportable, has acceptable enzyme stability compared to leucocyte and/or

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fibroblast culture, it is recommended as a first screening test. However the false positive rate with DBS sample is higher compared to other samples (1-3). DBS is a sample from the total blood of subjects, it contains erythrocytes, leucocytes, thrombocytes which might affect the enzyme activity measurement. Raghavan et al. (4) firstly pointed out that the total specific activity of a Gaucher heterozygote might lead to misdiagnosis due to the variation in enzyme activities in different types of leucocyte. Since then, many studies have been conducted to produce greater test accuracy and to increase the diagnostic efficacy of DBS samples (5-7). The strict control of storage conditions (such as humidity, temperature and so on), the correct preparation of the sample, sample blank correction and participation in quality control programs are recommended by many authors in order to validate these methods. Recently, the specificity and sensitivity of DBS enzyme assays to diagnose LSD have been reported as 95.2% (92-96%) and 85% (64-100%) respectively (5,6). Even though all above mentioned factors were controlled, the false positive rates are still high because of individual factors, such as pseudo deficiency due to isozymes and so on. The determination of enzyme activities in leucocyte samples were accepted as the gold standard test because of the corrected calculation of enzyme activity regarding protein levels in leucocytes and its high sensitivity and specificity. Although some authors investigated the factors which affect lysosomal enzyme activity in leucocyte and the enzyme activities in different types of leucocytes (4,8-10), there is no data on the effect of hemogram parameters, especially leucocyte numbers, on the enzyme activities in DBS samples. We investigated any possible effects of these parameters on enzyme activity measurements in dried blood samples in a retrospective study in order to explain the false positive rate due to individual factors.

Materials and Methods

This study was approved by the Local Ethics Committee of Ege University (approval number: 16-8/2). Data was collected from the subjects for whom hematological parameters (hemoglobin, hematocrit, leucocyte, neutrophil, lymphocyte and thrombocyte) were available in the database of Ege University Hospital. Totally 1500 subjects clinically suspected had been screened for LSD from January 2015 to September 2016. Clinically and genetically verified patients were excluded. Two hundred sixty three subjects of this population had hematologic test results. Lysosome enzyme activities had been determined by the ultra-high pressure liquid chromatography-tandem mass spectrometer (MS/MS) method (sphingomyelinase and galactocerebrosidase activities) and fluorometric method (α-galactosidase, α-glycosidase, glycocerebrosidase activities) and hematological parameters had been determined by routine laboratory methods.

Statistical Analysis

All data were evaluated using the SPSS 22.0 statistics program and comparisons were made using the Student t-test, correlations were calculated using the Pearson test.

Results

The general characteristics of population are presented in Table I. Glycocerebrosidase enzyme activity showed a positive correlation with the total number of leukocytes and the number of neutrophils (Figure 1a). Sphingomyelinase enzyme activity showed a positive correlation with the total number of leukocytes and the number of lymphocytes (Figure 1b). Alpha galactosidase enzyme activity showed a positive correlation with the total number of leukocytes and the number of lymphocytes as well as the number of neutrophils (Figure 1c). Interestingly, alpha galactosidase activity correlated with the number of thrombocytes (r=0.373, p=0.000). Alpha glycosidase activity positively correlated with the total number of leucocytes, the number of neutrophils and the number of lymphocytes and the number of thrombocytes (r=0.197, p=0.007) (Figure 1d). Galactocerebrosidase activity positively correlated with the total number of leucocytes, the number of neutrophils and the number of thrombocytes (r=0.142, p=0.001) (Figure 1e). There was no correlation between enzyme activities and hemoglobin/hematocrit levels. Subjects (n=53) with glycocerebrosidase enzyme activity lower than 0.9 nmol/mL/h (which is the cut off value to recall the patients) exhibited a significantly lower number for leukocyte (p=0.02) and lymphocyte (p=0.015) compared to those of subjects with enzyme activity higher than 0.9. Subjects (n=19) with sphingomyelinase enzyme activity lower than 3.7 nmol/mL/h (which is the cut off value to recall the patients) exhibited a significantly lower number of lymphocyte (p=0.015) compared to those of subjects with enzyme activity higher than 3.7. Subjects (n=23) with

Table I. General characteristic of study population

	n	Mean ± SD
Gender (male/female)	263	128/135
Glycocerebrosidase (nmol/mL/h)	263	1.87±1.44
α glycosidase (nmol/mL/h)	198	2.62±1.34
α galactosidase (nmol/mL/h)	213	6.03±3.67
Sphingomyelinase (nmol/mL/h)	181	10.32±7.81
Galactocerebrosidase (nmol/mL/h)	170	1.33±0.89
Hemoglobin (g/dL)	263	11.94±1.89
Hematocrite (%)	263	36.71±5.43
Total leucocyte/mm^3	263	8.916±3.748
Neutrophil number/mm^3	263	3.861±2.261
Lymphocyte number/mm^3	263	3.846±2.482
Thrombocyte number/mm^3	263	291.839±122.747

SD: Standard deviation

alpha galactosidase enzyme activity lower than 2.2 nmol/mL/h (which is the cut off value to recall the patients) exhibited a significantly lower number of thrombocyte (p=0.015) compared to those of subjects with enzyme activity higher than 2.2. Since there was only one subject with low alpha glycosidase activity and 3 subjects with low galactocerebrosidase activity according to their respective cut off limits, they could not be analyzed statistically. We recalculated the estimated leucocyte enzyme activities by dividing the enzyme activities of DBS by the leucocyte number and the reference intervals of estimated leucocyte enzyme activities for healthy population were determined using the nonparametric method according to the procedure recommended by the International Federation of Clinical Chemistry and National Committee for Clinical Laboratory Standards (11). A one-sided reference region (5th percentile) at the lower end was accepted as the decision-making limit and data was re-evaluated with regard to this limit of estimated leucocyte enzyme activities. When we recalculated the glycocerebrosidase enzyme activities with regard to the leucocyte number, the false positive number declined from 53 to 12. While 53 of subjects had had lower enzyme activities than the cut off values, only 12 of these 53 subjects had lower "estimated leucocyte glycocerebrosidase activity" (the glycocerebrosidase/leucocyte number) after making a correction based on the leucocyte number. Similarly, of the 19 subjects who originally had low sphingomyelinase activity, only 8 still had low estimated leucocyte sphingomyelinase activity, 11 of the 19 subjects exhibited normal estimated leucocyte sphingomyelinase activity. Similarly, of the 23 subjects with low alpha galactosidase activity, only 9 still had low estimated leucocyte alpha galactosidase activity, 14 of 23 subjects exhibited normal estimated leucocyte alpha galactosidase activity. From these data, the false positive rates for glycocerebrosidase, sphingomyelinase and alpha galactosidase decreased via a correction activity based on the leucocyte number from 20%, 10.5% and 10.8% to 4.5%, 4.4% and 4.2%, respectively.

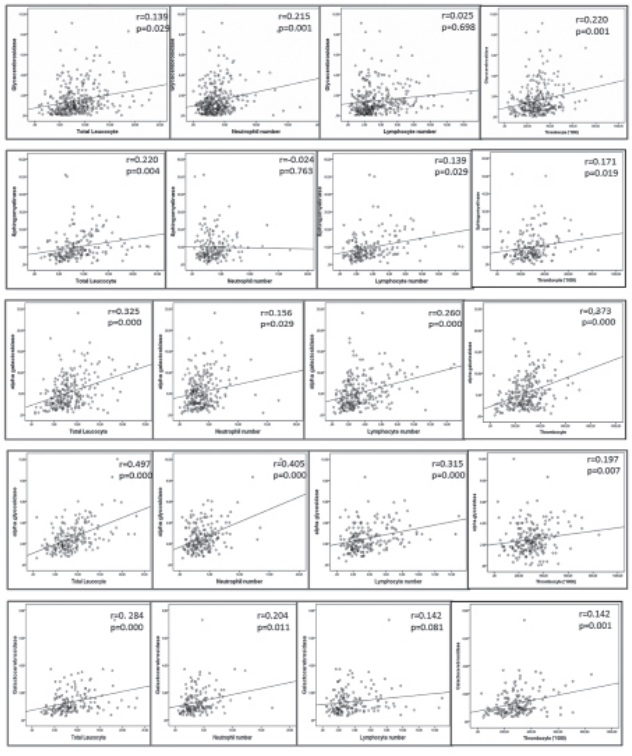

Figure 1. a-e. a) Correlation between the glycocerebrosidase activity and leucocytes, thrombocyte, b) Correlation between the Sphingomyelinase activity and leucocytes, thrombocyte, c) Correlation between alpha galactosidase activity and leucocytes, thrombocyte, d) Correlation between alpha glycosidase activity and leucocytes, thrombocyte, e) Correlation between galactocerebrosidase activity and leucocytes, thrombocyte

Discussion

Although DBS samples are the most suitable material for screening and diagnosis of LSD, interferences related to sample quality might lead to misdiagnosis and/or false positive/negative test results. We investigated the relationship between the hematologic parameters of patients and enzyme activity in DBS materials. Since we corrected the enzyme activity calculation using sample blank to prevent hemoglobin interferences, there was no correlation between the hematocrit/hemoglobin levels and enzyme activity (12). In this study, we showed that all enzyme activities in DBS are closely correlated with the total number of leucocyte, since leucocytes are the main source of lysosomal enzymes. While glycocerebrosidase and galactocerebrosidase presented a positive correlation with the number of neutrophils, sphingomyelinase showed a positive correlation with the number of lymphocytes. Alpha galactosidase and alpha glycosidase enzyme activity were correlated both with neutrophils and lymphocytes. Similarly, Nakagawa et al. (13) found that β-glucosidase in all cell types is mainly membrane bound and it is stimulated by taurocholate in granulocytes, monocytes and lymphocytes, so they suggested that variations in the total number of leukocytes and in the relative proportion of their various cell types might lead to inconsistent or unreliable values for enzyme activity in the diagnosis of LSD and in carrier detection. Daitx et al. (14) observed no correlation between the enzyme activities in DBS and in leukocytes for the whole population because of the corrected calculation of enzyme activity regarding protein levels in leucocytes and the inhibition of alpha-galactosidase B isozyme activity by N-acetyl-D-galactosamine in DBS samples but not inhibition of isozyme in leucocyte samples (14). Elbin et al. (7) demonstrated that the enzyme activities can be affected by inadequate blood spot processing especially by an incomplete mixing of blood before spotting (7) in accordance with these observations, we showed that the enzyme activities in DBS sample are closely correlated with the number of leucocytes of patients. From this data, we concluded that the calculation of enzyme activity

with regard to the number of leucocytes might produce more reliable results and might help to decrease the false positive rate. However, it is crucially important to decide the cut off levels of enzymes for diagnosis and for re-testing. Recently, selective screening based on the main clinical findings in LSD has been recommended in the diagnosis of adult patients. Anemia and chronic renal insufficiency are common in Fabry patients and the screening studies among dialysis patients and/or anemia were conducted. Kleinert et al. (15) showed a high prevalence of anemia in 345 adults with Fabry disease from the international database, the Fabry Outcome Survey and proposed kidney failure, heart failure and/or inflammation as potential causes of anemia in Fabry disease (15). Although the main cause of anemia in Fabry disease patients is not clearly understood, it is speculated that the reasons were bone marrow accumulation of globotriaosylceramide or renal insufficiency. However, anemia might be proposed as a parameter to promote selective screening for Fabry disease from this data, the conflicting effect of anemia on enzyme activity in DBS sample should be taken into account for screening studies. Our data indicated that the enzyme activity in dried blood samples including low leucocyte number might be found lower than reference intervals resulting in false positive diagnosis. Therefore, we suggest that the laboratory scientists should evaluate the number of leucocyte while interpreting data. From this data, we concluded that the calculation of enzyme activity with regard to the number of leucocytes might achieve more reliable results and might be helpful in decreasing the false positive rate.

Study Limitations

Since patients with LSD were excluded from this study, we could not calculate the false-negative rate and also the specificity and sensitivity of estimated leucocyte enzyme activity.

Conclusion

DBS is the most advantageous matrix for the screening for LSD. However, many factors including hemoglobin levels, transport conditions and/or leukocyte levels might affect the enzyme activities in DBS. Our study showed that a low number of leukocytes is one of the critical factors affecting enzyme activity. Therefore, it is crucially important to interpret the data taking into account the leukocyte number in order to decrease the number of recall patients and the false-positive ratio.

Ethics

Ethics Committee Approval: This study was approved by Local Ethics Committee of Ege University (approval number: 16-8/2).

Informed Consent: Retrospective study.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.K.U., M.Ç., Concept: E.Y.S., Design: E.Y.S., Data Collection or Processing: M.D., E.Y.S., Analysis or Interpretation: E.Y.S., S.K.U., M.Ç., Literature Search: M.D., E.Y.S., Writing: E.Y.S., S.K.U., M.Ç.

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References

1. Kingma SDK, Bodamer OA, Wijburg FA. Epidemiology and diagnosis of lysosomal storage disorders; challenges of screening. Best Pract Res Clin Endocrinol Metab 2015;29:145-57.
2. Reusera AJ, Verheijena FW, Balib D, et al. The use of dried blood spot samples in the diagnosis of lysosomal storage disorders-Current status and perspectives. Mol Genet Metab 2011;104:144-8.
3. de Jesus VR, Zhang XK, Keutzer C, et al. Development and evaluation of quality control dried blood spot materials in newborn screening for lysosomal storage disorders. Clin Chem 2009;55:158-64.
4. Raghavan SS, Topol J, Kolodny EH. Leukocyte ,8-Glucosidase in Homozygotes and Heterozygotes for Gaucher Disease. Am J Hum Genet 1980;32:158-73.
5. Verma J, Thomas DC, Kasper DC, et al. Inherited Metabolic Disorders: Efficacy of Enzyme Assays on Dried Blood Spots for the Diagnosis of Lysosomal Storage Disorders. JIMD Rep 2017;31:15-27.
6. Stroppiano M, Calevo MG, Corsolini F, et al. Validity of β-D-glucosidase activity measured in dried blood samples for detection of potential Gaucher disease patients. Clin Biochem 2014;47:1293-6.
7. Elbin CS, Olivova P, Marashio CA, et al. The effect of preparation, storage and shipping of dried blood spots on the activity of five lysosomal enzymes. Clin Chim Acta 2011;412:1207-12.
8. Olivova P, Veen K, Van Der, et al. Effect of sample collection on α-galactosidase A enzyme activity measurements in dried blood spots on fi lter paper. Clin Chim Acta 2009;403:159-62.
9. Burin M, Dutra-Filho C, Brum J, et al. Effect of collection, transport, processing and storage of blood specimens on the activity of lysosomal enzymes in plasma and leukocytes. Braz J Med Biol Res 2000;33:1003-13.
10. Lukacs Z, Cobos PN, Mengel E, et al. Diagnostic efficacy of the fluorometric determination of enzyme activity for Pompe disease from dried blood specimens compared with lymphocytes-possibility for newborn screening. J Inherit Metab Dis 2010;33:43-50.
11. National Committee for Clinical Laboratory Standards. How to define and determine reference intervals in the clinical laboratory: approved guideline, 2001. NCCLS document C28-A and C28-A2, Villanova, PA, NCCLS.

12. Sözmen EY. Letter to Editor. Sample blank subtraction outreachs Hemoglobin interferences in flurorometric methods for DBS. Mol Genet Metab 2012;105:530.
13. Nakagawa S, Kumin S, Nitowsky HM. Studies on the activities and properties of lysosomal hydrolases in fractionated populations of human peripheral blood cells. Clin Chim Acta 1980;101:33-44.
14. Daitx VV, Mezzalira J, Pereira M, Goldim DS, Coelho JC. Comparison between alpha-galactosidase A activity in blood samples collected on filter paper, leukocytes and plasma. Clin Biochem 2012;45:1233-8.
15. Kleinert J, Ois Dehout F, Schwarting A, et al. Anemia is a new complication in Fabry disease: Data from the Fabry Outcome Survey. Kidney Int 2005;67:1955-60.

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Original Article

Initial and Final Status of the Patients with Niemann Pick A and B: Ege University Experience

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ABSTRACT

Aim: Niemann-Pick disease (NPD) is a lysosomal storage disease caused by an insufficient activity of acid sphingomyelinase (ASM) resulting in the accumulation of sphingomyelin. Type A is an infantile neurovisceral fatal form characterized by hepatosplenomegaly and rapidly progressive neurological deterioration, while the Type B non-neuronopathic disease presents visceral form and sufferers usually survive into adulthood.

Materials and Methods: Here we present clinical and molecular findings for 19 patients with NPD A/B.

Results: Nineteen patients with ASM deficiency were enrolled in our study. Nine of them were female and ten patients were male. The median age of the patients was 7.5 years (minimum-maximum: 1-57 years), the median age at diagnosis was 3 years (minimum-maximum: 6 months-56 years). The median length of the follow up period was 4.07±3.8 years (range: 1 month-14 years). Eighteen patients had hepatosplenomegaly, one patient had splenomegaly. Pulmonary involvement was detected in 10 patients. Six patients died during follow up.

Conclusion: Patients with Niemann Pick A/B have a high mortality and morbidity rate. There is a need for a safe and effective therapy for patients with NPD A/B to reduce splenomegaly, to improve liver and respiratory function and to reduce the rate of mortality and morbidity.

Keywords: Hepatosplenomegaly, interstitial pulmonary disease, cytopenia, acid sphingomyelinase

Introduction

Niemann-Pick disease (NPD) is a lysosomal storage disease caused by the insufficient activity of acid sphingomyelinase (ASM) resulting in the accumulation of sphingomyelin in the monocyte-macrophage system. Type A (MIM #257200) is an infantile neurovisceral fatal form characterized by hepatosplenomegaly and rapidly progressive neurological deterioration while Type B (MIM #207616) is non-neuronopathic form. Type B is characterized by visseromegaly and sufferers usually survive into adulthood (1). The NPD-Type B (NPD-B) phenotypic spectrum among individuals varies widely and the age of onset may be from early childhood to adulthood. Hepatosplenomegaly is the most frequent clinical finding in NPD-B and secondary hypersplenism, growth restriction, pulmonary involvement and mild liver failure can also be seen (2,3) The most consistent laboratory finding is an abnormal lipid profile (i.e. high triglycerides and low density lipoprotein-cholesterol, low high density lipoprotein-cholesterol) and a history of the coronary arterial disorder may also be found (4,5). The lungs are frequently affected in NPD-B patients. In some patients, eye examination revealed distinct cherry red spot. There may also be a reddish-brown halo surrounding the macula in the eyes of some patients. Other common findings are fatigue, bone and joint pain and osteopenia. Thrombocytopenia and leukopenia typically worsen over time. NPD-A patients exhibit hepatosplenomegaly and failure to thrive within the first year

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of life. A cherry red spot in the eye is present in 50% of these patients. Type A is characterized by a rapidly progressive neurodegeneration with profound hypotonia (6). Patients with intermediate findings between NPD-A and NPD-B have been described (6). In 1967 Kampine et al. (7) described the first enzymatic determination of these disorders by determining enzyme activities in peripheral blood leukocytes. The *ASM* gene (*SMPD1*; MIM #607608) contains 6 exons and is located on chromosome 11p15.1-11p15.4 (1). It is well documented that Type A-causing *SMPD1* mutations occur more frequently among patients with Ashkenazi Jewish heritage than in the general population (8). Our aim is to discuss the clinical and molecular findings of patients with NPD A/B who were followed up at Ege University Faculty of Medicine, Pediatric Metabolism and Nutrition Unit.

Materials and Methods

Nineteen patients who were diagnosed with Niemann Pick A and B at Ege University Faculty of Medicine, Department of Pediatric Metabolism and Nutrition Unit were included in this study. The patients' demographical features including age, sex, age at diagnosis, clinical findings; ophthalmological examination, organomegaly, pulmonary and gastrointestinal complications and their surgical histories were noted. We collected biochemical data to determine the function of their organs (liver function, renal function, lipoprotein levels). Liver and/or bone marrow biopsy results which had been done during the diagnostic period and acid sphingomyelinase enzyme levels were evaluated. *SMPD1* gene analysis was also recorded. Where available, radiological information was also included. A consent form was filled out by all participants.

Statistical Analysis

Statistical analysis was performed using "SPSS for Windows 22" software. The descriptive values of demographic and clinical parameters were analysed. Categorical variables are given as case number and percentage. Descriptive statistics are shown as mean ± standard deviation (SD) or median (minimum-maximum) for numeric variables.

Table I. Demographical and clinical findings of the patients with acid sphingomyelinase deficiency

Patient no	Age (years)	Age at diagnosis	Gender consanguinity	Current status	Follow up period (years)	Clinical findings	Clinical based NPD Type
1	7.5	1 years	M/first degree cousin	Alive	7.6	HSM, bleeding CRS, ILD	B
2	7	1.5 years	F/first degree cousin	Alive	7	HSM	B
3	-	3 months	F/first degree cousin	Deceased (16 months)	1.3	HSM, ILD, DD	A
4	21	9 years	F/first degree cousin	Alive	14	HSM, bleeding, ILD, psychiatric symptoms	B
5	*	5.5 years	M/first degree cousin	Deceased	8	HSM, bleeding, ILD	B (severe)
6	*	3 years	M/first degree cousin	Deceased	8	HSM, bleeding, ILD	B (severe)
7	21	4 years	M/first degree cousin	Alive	7.8	HSM	B
8	16	13 years	F/first degree cousin	Alive	4	HSM, ILD	B
9	28	25 years	F/first degree cousin	Alive	3.8	HSM, ILD	B
10	57	56 years	F/first degree cousin	Alive	2	HSM, bleeding, CRS	B
11	19.5	19 years	M/first degree cousin	Alive	0.3	HSM, ILD	B
12	1	7 years	M/first degree cousin	Alive	0.5	HSM, CRS, ILD, DD	A/B
13	7	6.5 years	M/first degree cousin	Alive	0.5	HSM	B
14	6	3 years	F/first degree cousin	Alive	3.5	SM	B
15	-	6 months	M/third degree	Deceased (3 years old)	1	HSM, bleeding, CRS, DD	B (severe)
16	-	1 years	M/first degree cousin	Deceased	0.6	HSM, CRS, DD	A
17	-	6 months	F/first degree cousin	Deceased	0.08	HSM, CRS, DD	A
18	7.5	6 months	F/none	Alive	2	HSM	B
19	9	3.5 years	M/third degree	Alive	5.5	HSM, ILD	B

HSM: Hepatosplenomegaly, SM: Splenomegaly, CRS: Cherry red spot, ILD: Interstitial lung disease, DD: Developmental delay, NPD: Niemann-Pick disease, F: Female, M: Male
*They died and ages at last-visit were 13 and 11 years respectively

Results

Nineteen patients with NPD-A and NPD-B were enrolled in our study. Nine of them were female, ten patients were male. The median age of the patients was 7.5 years (minimum-maximum: 1-57 years) the median age at diagnosis was 3 years (minimum-maximum: 6 months-56 years). The median length of the follow up period was 4.07±3.8 years (range: 1 month-14 years). Two patients had low weight SD score (SDS) (-2.15 and -2.32), three patients had growth retardation, height SDS were between -2.31 and -5.2. Five patients had low weight and height SDS. The weight SDS ranged from -2.05 to -3.06 and the height SDS ranged from -3.03 to -6.7. Six (31.5%) patients had bleeding (minor mucosal bleeding) and fifteen (78.9%) patients had abdominal distension and pain. One (5.2%) of our patients had joint pain and a history of the treatment at rheumatology clinics. Eight (42.1%) patients had a history of recurrent infections such as bronchiolitis, pneumonia and otitis media. Two (10.4%) of these (patient 1 and 12) have hearing loss due to recurrent otitis media. Hepatosplenomegaly was detected in 18 (94.7%) patients. Additionally, one (5.2%) of the patients had splenomegaly. Cherry red spot was observed in 6 (31.5%) patients. Pulmonary involvement was determined in 10 (52.6%) patients via clinical and radiological findings. Three (15.7%) of our patients were diagnosed in adulthood but had a history of abdominal distension and splenomegaly in childhood. Five (26.3%) patients had developmental delay. The demographical, clinical and diagnostic laboratory findings of the patients are detailed in Table I. Clinical signs and symptoms are given in Figure 1. Liver biopsies were performed on 5 patients and hepatic steatosis and liver fibrosis were detected. Bone marrow biopsies were performed on 10 patients and lipid-laden foam cells were detected. Osteoporosis was detected in one patient. Acid sphingomyelinase enzyme levels were low in all patients. *SMPD1* gene analysis was performed on 17 patients in the study. *SMPD1* analysis for patient 6 could not be performed. The enzyme levels and *SMPD1* gene analysis of the patients are detailed in Table II.

Anemia was detected in 3 patients, thrombocytopenia was detected in 1 patient, bicytopenia (anemia + thrombocytopenia) was detected in 3 patients and pancytopenia was detected in 2 patients as initial findings. There was hypertriglyceridemia

Table II. Acid sphingomyelinase levels and *SMPD1* gene analyses of the patients with Niemann-Pick disease Type A and Type B

Patient no	Acid sphingomyelinase (normal)	*SMPD1* gene analysis	Genomic mutation amino acid change
1	0.003 nmol/17 h/mg prot (7.7±3.08)	c.409T>C/c.409T>C	p.L137P/p.L137P
2	1.28 nmol/17 h/mg prot (7.7±3.08)	c.409T>C/c.409T>C	p.L137P/p.L137P
3	1.2 nmol/17 h/mg prot (7.7±3.08)	c.567delT/c.567delT	p.P189PfsX65/p.P189PfsX65
4	0.5 nmol/mL/h (3.7-21)	c.409T>C/c.409T>C	p.L137P/p.L137P
5*	2.9 nmol/h/mg prot (84-270)	c.409T>C/c.409T>C	p.L137P/p.L137P
6*	2.3 nmol/h/mg prot (84-270)	NA	NA
7	0.2 µmol/L/h (1-10)	c.482T>C/c.482T>C	p.L161P/p.L161P
8	0.1 nmol/mL/h (1.3-15)	c.409T>C/c.409T>C	p.L137P/p.L137P
9	0.45 nmol/mL/h (3.7-21)	c.409T>C/c.409T>C	p.L137P/p.L137P
10	0.08 nmol/mL/h (1.3-15)	c.847G>A/c.847G>A	p.A283T/p.A283T
11	0.2µ mol/L/h (>0.9)	c.1652T>C/c.1652T>C	p.L551P/p.L551P
12	0.06 nmol/mL/h (1.3-15)	c.573delT/c.573delT	p.S192AfsX65/p.S192AfsX65
13	0.2 nmol/mL/h (1.3-15)	NA	NA
14	0.18 nmol/17 h/mg prot (7.7±3.08)	c.528G>C/c.533T>A	p.W176C/p.I178N
15	0.08 nmol/17 h/mg prot (7.7±3.08)	c.1755delC/c.1755delC	p.P585PfsX24/p.P585PfsX24
16	0.46 nmol/17 h/mg prot (7.7±3.08)	c.567delT/c.567delT	p.P189PfsX65/p.P189PfsX65
17	0.272 µmol/L/h (1-10)	c.567delT/c.567delT	p.P189PfsX65/p.P189PfsX65
18	0 nmol/17 h/mg prot (7.7±3.08)	c.409T>C/c.1262A>G	p.L137P/p.H421R
19	0.66 nmol/mL/h (1.3-15)	c.1552T>C/c.1552T>C	p.L549P/p.L549P

*Sibling, NA: Not applicable

in 9 patients and hypercholesterolemia in 9 patients. Nine patients had high transaminase levels. During follow up, 8 patients' transaminase levels returned to normal ranges. Complete blood count and other biochemical investigations are shown in Table III. Chitotriosidase activity measurements were performed on 12 patients. The mean chitotriosidase level was 179.3±234.3 nmol/mL/h (normal range: 0-38, minimum-maximum: 8.9-818) and high chitotriosidase levels were detected in 6 patients. Patient 4 developed psychiatric signs and was treated with antipsychotic drugs. Patient 1 had a history of recurrent febrile episodes and was diagnosed with Familial Mediterranean Fever. A homozygous M694V mutation was detected under *MEFV* gene analysis. He was treated with colchicine. During follow up, bone marrow transplantation was planned for him and he underwent partial splenectomy surgery. Due to donor problems and his parents' decision, Bone marrow transplantation (BMT) was not performed. Pulmonary involvement was frequent in our patients. High-resolution computed tomography analysis was performed on 10 patients and a reticulonodular interstitial pattern was detected. Echocardiography revealed mitral insufficiency in three patients and aortic insufficiency in one patient. Coronary arterial disease was not detected. Six patients died during follow up. The latest status for patient 6 was unavailable at the time of writing. Patient 3 died at the age of 16 months; she was clinically based NPD-A. She had severe developmental delay combined with massive hepatosplenomegaly. She had severe interstitial lung disease and died due to pulmonary complications. Her cranial magnetic resonance imaging investigation revealed bilateral cerebral hemisphere diffuse atrophy. Patient 5 and 6 discontinued their follow up. Their ages at last-visit were 13 and 11 years respectively. We subsequently found out that that they had died. Patients 15 and 17 died during follow up. Patient 15 had developmental delay combined with hepatosplenomegaly and died at home due to an aspiration after vomiting. Patient 16 and 17 discontinued their follow up and were subsequently found to have died.

Discussion

Acid sphingomyelinase deficiency is a rare disease with an estimated incidence of 0.4 to 0.6 in 100.000 new-borns (6). We have no data for the incidence of ASM deficiency in our country. Due to the high number of consanguineous marriages, a high incidence is suspected. In our study, consanguinity was recorded in 18 patients. Clinically, ASM deficiency has been classified as NPD-A and NPD-B based on the presence or absence of neurological involvement respectively (6). In our study, 4 of our patients had the clinical signs of NPD-A and the other 15 patients had the clinical findings of NPD-B. The first symptom in most patients with NPD-A is hepatosplenomegaly which is typically observed by the age of 3 months. (9). Likewise, hepatosplenomegaly is a consistent feature of NPD-B patients (10). All patients in our study had splenomegaly. Among patients with NPD-B, there is a broad range of disease severity. Age at clinical presentation can range from early childhood to the fourth or fifth decades of life (6,9). Three of our patients were diagnosed in adulthood but two of them had a history of abdominal distension and splenomegaly in childhood. The diagnosis was delayed. In the literature; growth retardation was shown in 29% of the patients with NPD-B and was particularly prominent in adolescents. Also, it was shown that weight Z scores were >-2 in 15% of NPD-B patients. We found similar findings; two patients had low weight SDS, three patients had low height SDS and five patients had both low weight and height SDS. NPD-B is most commonly associated with pulmonary involvement, expressed through interstitial lung disease (10,11). In our study, 10 patients had interstitial lung disease. Patient 1 had recurrent pulmonary infections, but during follow up, symptoms became less severe. Patient 3 had a history of respiratory insufficiency. In the literature, several cases with NPD-B presented with evere liver disease (e.g. hepatic failure) were reported (12). In this study, nearly half of the patients had high transaminase levels. One patient died due to liver failure and 3 others had liver failure.

Table III. Initial laboratory studies of the patients with Niemann-Pick disease Type A and Type B

Laboratory study	Mean ± SD	Range	Abnormal (%)
Hemoglobin, g/L	11.02±1.8	7.2-14.2	8 (42)
Hematocrit, %	32.4±5.2	21-41	8 (42)
White blood cells x103/L	8642.6±4888.8	3210-20300	2 (10.5)
Platelet, <109/L	212578±79944.6	52000-311000	6 (31.5)
Total cholesterol mg/dL	204.5±55.3	107-293	9 (47.3)
HDL cholesterol mg/dL	26.4±13.9	8-51	4 (21)
LDL cholesterol mg/dL	133.4±53.6	42-204	9 (47.3)
Triglycerides mg/dL	204.1±85.5	88-364	9 (47.3)
ALT, IU/L	137.4±199.3	10-849	9 (47.3)
AST IU/L	191.3±266.8	16-947	9 (47.3)
Total bilirubin mg/dL	1.2±1.9	0.14-7.2	2 (10.5)

SD: Standard deviation, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, ALT: Alanine transaminase, AST: Aspartate transaminase

Mcgovern et al. (10) showed that coronary artery disease is a more common manifestation than previously reported. Additionally, Ishii et al. (13) described two young sisters with NPD-B who had myocardial dysfunction refractory to treatment. We did not detect serious cardiac involvement in our patients. It should be taken into account that only three patients were in adulthood. Acid sphingomyelinase levels were low in all our patients. This diagnosis was confirmed with *SMPD1* gene analysed in 17 patients. Bone marrow biopsies were performed on 10 patients and lipid-laden foam cells were detected. The detection of vacuolated cells in peripheral blood smears or bone marrow is indicative of NPD but not exclusive to it. The diagnosis should be confirmed by enzymatic analysis and *SPMD1* gene analysis (1). Bone marrow biopsies were performed on almost half of the patients although the diagnosis can be made by enzyme and molecular analysis. Keeping NPD in mind for the differential diagnosis of patients with organomegaly, cytopenia and/or pulmonary involvement may prevent the use of unnecessarily invasive procedures in the diagnostic approach. To date, >180 mutations have been found within the *SMPD1* gene, which cause NPD-A, NPD-B. Aykut et al. (14) found one novel mutation c.1755delC (p.585PfsX24) in one of our patients and reported so in 2013. The most common mutations were previously known: c.409T>C (p.L137P) homozygous missense mutations. The L137P mutation has been linked to a less severe form NPD-B (15). In our study, patients with c.409T>C (p.L137P) also exhibited less severe symptoms. Although the p.L137P mutation is related to a less severe form, patient 5 died during adolescence due to liver failure and his brother also died prematurely (patient 6). The 573delT (p.P189fsX65) mutation had been previously identified by Gluck et al. (16). This mutation induces early onset form NPD-A (16). Manshadi et al. (17) also identified a patient with a 573delT homozygous mutation. Unusually, patient 12 had a homozygous 573delT (p.S192AfsX65) mutation and he has mild developmental delay but does not exhibit the clinical signs of early onset NPD-A. Patients with the intermediate variation between Types A and B NPD have been described (6). Patient 12 seems to be of an intermediate type. The most common Type B mutations in Turkish patients are L137P, L549P and fsP189 (15). Similarly to the literature, in total, 10 patients in our study had these mutations. Patient 3 had a common mutation for Type B but her clinically based type was similar to Type A. The patient with an A283T mutation, who was diagnosed at the age of 56 years, was clinically NPD-B. Patient 18 had compound heterozygous mutations for p.H421R and p.L137P. Although p.H421R is known to be a severe one, the combination with the L137P mutation reduced the phenotypic severity as previously reported by Aykut et al. (14). In our study, those patients with the same homozygous L137P mutations, had different clinical severity (patient 1, 2, 4, 5, 8, 9) such as patient 5, who had severe clinical findings. Some patients with NPD-B may develop life threatening complications including liver failure, hemorrhage, pulmonary infections and splenic rupture. The most common causes of disease related morbidity and mortality are respiratory and liver failure (18). Similar to our study, the deceased patients had respiratory insufficiency and patient 3 had liver failure.

BMT has been performed on some NPD patients (1). A reduction in liver and spleen size have been demonstrated. In our study, we had planned BMT for patient 1. He had a partial splenectomy operation before the planned BMT. However, during follow up, we could not perform BMT due to donor problems and his parents' subsequent decision to postpone BMT. Today, clinical trials are being conducted to determine the safety and efficacy of enzyme replacement therapy for NPD-B patients (19). There is a need for a safe and effective therapy for patients with NPD-B to reduce splenomegaly, improve liver function, decrease respiratory dysfunction and reduce mortality and morbidity.

Study Limitations

There are several limitations in this study. We investigated a small group of patients with NPD consisting of children and adults. Also, we determined the lung involvement of the patients but could not perform any functional tests.

Conclusion

Serial findings of 19 patients with ASM deficiency gave information about the natural history of the disease. The most common sign of NPD is hepatosplenomegaly. Pulmonary involvement is important for the disease's mortality and morbidity. Patients need more efficient therapies to reduce organomegaly and to improve liver and respiratory functions.

Ethics

Informed Consent: A consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.C., M.Ç., Concept: E.C., S.K.U., Design: E.C., M.Ç., Data Collection and Processing: F.Ö., H.O., Analysis and Interpretation: E.S., Literature Search: H.Y., E.E., Writing: E.C.

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References

1. Schuchman EH, Desnick RJ. Niemann-Pick disease types A and B: acid sphingomyelinase deficiencies. In: Scriver CR, Beaudet AL, Sly WS, et al, (eds). The Metabolic and Molecular Bases of Inherited Disease. 8th ed. New York, NY: McGraw–Hill 2001:3589-610.
2. Wasserstein MP, Larkin AE, Glass RB, Schuchman EH, Desnick RJ, McGovern MM. Growth restriction in children with type B Niemann-Pick disease. J Pediatr 2003;142:424-8.
3. Simonaro CM, Desnick RJ, McGovern MM, Wasserstein MP, Schuchman EH. The demographics and distribution

of type B Niemann-Pick disease: novel mutations lead to new genotype/phenotype correlations. Am J Hum Genet 2002;71:1413-9.

4. McGovern MM, Pohl-Worgall T, Deckelbaum RJ, et al. Lipid abnormalities in children with types A and B Niemann Pick disease. J Pediatr 2004;145:77-81.
5. Lee CY, Krimbou L, Vincent J, et al. Compound heterozygosity at the sphingomyelin phosphodiesterase-1 (SMPD1) gene is associated with low HDL cholesterol. Hum Genet 2003;112:552-62.
6. Schuchman EH, Desnick RJ. Types A and B Niemann-Pick disease. Mol Genet Metab 2017;120:27-33.
7. Kampine JP, Brady RO, Kanfer JN, Feld M, Shapiro D. Diagnosis of gaucher's disease and niemann-pick disease with small samples of venous blood. Science 1967;155:86-8.
8. ACOG Committee on Genetics. ACOG Committee Opinion No. 442: Preconception and prenatal carrier screening for genetic diseases in individuals of Eastern European Jewish descent. Obstet Gynecol 2009;114:950-3.
9. Wasserstein MP, Aron A, Brodie SE, Simonaro C, Desnick RJ, McGovern MM. Acid sphingomyelinase deficiency: prevalence and characterization of an intermediate phenotype of Niemann-Pick disease. J Pediatr 2006;149:554-9.
10. McGovern MM, Wasserstein MP, Giugliani R, et al. A prospective, cross-sectional survey study of the natural history of Niemann-Pick disease type B. Pediatrics 2008;122:341-9.
11. von Ranke FM, Pereira Freitas HM, Mançano AD, et al. Pulmonary Involvement in Niemann-Pick Disease: A State-of-the-Art Review. Lung 2016;194:511-8.
12. Dardis A, Zampieri S, Filocamo M, Burlina A, Bembi B, Pittis MG. Functional in vitro characterization of 14 SMPD1 mutations identified in Italian patients affected by Niemann Pick Type B disease. Hum Mutat 2005;26:164.
13. Ishii H, Takahashi T, Toyono M, et al. Acid sphingomyelinase deficiency: cardiac dysfunction and characteristic findings of the coronary arteries. J Inherit Metab Dis 2006;29:232-4.
14. Aykut A, Karaca E, Onay H, et al. Analysis of the sphingomyelin phosphodiesterase 1 gene (SMPD1) in Turkish Niemann-Pick disease patients: mutation profile and description of a novel mutation. Gene 2013;526:484-6.
15. Simonaro CM, Desnick RJ, McGovern MM, Wasserstein MP, Schuchman EH. The demographics and distribution of type B Niemann-Pick disease: novel mutations lead to new genotype/phenotype correlations. Am J Hum Genet 2002;71:1413-9.
16. Gluck I, Zeigler M, Bargal R, Schiff E, Bach G. Niemann Pick Disease type A in Israeli Arabs: 677delT, a common novel single mutation. Mutations in brief no. 161. Online. Hum Mutat 1998;12:136.
17. Manshadi MD, Kamalidehghan B, Keshavarzi F, et al. Four novel p.N385K, p.V36A, c.1033-1034insT and c.1417-1418delCT mutations in the sphingomyelin Phosphodiesterase 1 (SMPD1) gene in patients with types A and B Niemann-Pick disease (NPD). Int J Mol Sci 2015;16:6668-76.
18. McGovern MM, Lippa N, Bagiella E, Schuchman EH, Desnick RJ, Wasserstein MP. Morbidity and mortality in type B Niemann-Pick disease. Genet Med 2013;15:618-23.
19. Wasserstein MP, Jones SA, Soran H, et al. Successful within-patient dose escalation of olipudase alfa in acid sphingomyelinase deficiency. Mol Genet Metab 2015;116:88-97.

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Original Article

Clinical Presentation and Follow Up of Patients with Mucopolysaccharidosis Type IVA (Morquio A Disease): Single Center Experience

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ABSTRACT

Aim: Mucopolysaccharidosis Type IVA (MPS IVA), Morquio A, is caused by the deficiency in lysosomal enzyme N-acetylgalactosamine-6-sulfate sulfatase. Multisystemic involvements include skeletal systems, pulmonary disease, valvular heart disease, hearing loss, mild hepatomegaly, corneal clouding, coarse facial features.

Materials and Methods: We retrospectively analyzed clinical and laboratory and follow up findings of our 25 patients with ministry for primary industries independent verification agency.

Results: Mean age of the patients was 14.9±7.05 (5.5-36 years). Mean age at diagnosis was 7.3±6.2 years (6 months-31 years). Female: male ratio was 13/12. All patients had skeletal manifestation and X-ray analysis demonstrated "dysostosis multiplex". Twelve patients (48%) had cardiac valve disease. Twenty three (92%) patients had corneal clouding, 15 (60%) patients had hearing loss and 9 (36%) had hepatomegaly. Six (24%) patients were unable to walk. Mean follow up period is 7.4 years ±3.5 years (3 months-17 years). Four patients have not visit our clinical for last ≥3 years. Three patients died during follow up.

Conclusion: MPS IVA is a severe disorder and is usually fatal in the second or third decade of life due to the complications of the disease. Early diagnosis of the patient became more important, because specific therapy with elasulphase alpha was approved recent years ago.

Keywords: MPS IVA, Morquio A, dysostosis multiplex, cardiac valve, corneal clouding

Introduction

Mucopolysaccharidosis Type IVA (MPS IVA or Morquio A disease; Online Mendelian Inheritance in Man 253000) was first described in 1929 by a pediatrician, L. Morquio in Montevideo (1). It is an autosomal recessive disorder caused by the deficiency of the lysosomal enzyme N-acetylgalactosamine-6-sulfate sulfatase (GALNS) (2). In the absence of this enzyme, the degradation of keratin sulfate (KS) and chondroitin 6-sulfate (C6S) is blocked, which leads to intracellular accumulation of glycosaminoglycans (GAG) in the lysosomes of various tissues, especially bone and cornea (2,3). The incidence of MPS IVA is estimated to be 1:201.000 ranges among various populations from 1 in 76.000 live births in Northern Ireland to 1 in 640.000 live births in Western Australia (4-6). Affected infants seem normal at birth but will progress disease signs within a few years. Over 70% of patients with MPS IVA have initial clinical manifestations of skeletal features within the first 2-3 years of life (7). These include striking short trunk dwarfism, odontoid hypoplasia, pectus carinatum, kyphosis, gibbus, scoliosis, genu valgum, coxa valga, flaring of the lower ribs, hypermobile joints, and abnormal gait with a tendency to fall. Patients with MPS IVA have preserved intelligence (7). The other problems include pulmonary disease, valvular heart disease, hearing loss, mild

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hepatomegaly, corneal clouding, and coarse facial features (8). The clinical manifestations of the disease and resulting impaired mobility can reduce the patient's ability to perform activities of daily living (9). Diagnosis is typically based on clinical examination, skeletal radiographs, and the enzymatic activity of GALNS in blood cells or fibroblast (7,10). *GLNS* gene located on chromosome 16q24:3, contains 14 exons (11,12). The management of Morquio A syndrome has typically been supportive and symptom-based. Specific enzyme replacement therapy (ERT) has recently become available. In 2014, the use of recombinant human GALNS, elosulfase alfa, was approved in the European Union, Canada, United States, Australia, and Brazil for the treatment of Morquio A syndrome. Elosulfase alfa is administered intravenously once-weekly at a dose of 2.0 mg/kg (13). Here we present our cases to describe an overview of the clinical manifestations, diagnosis, and management of patients with MPS IVA.

Materials and Methods

Twenty-five patients who were diagnosed with MPS IVA between 1995 and 2017 were included. Patients' demographical features including age, sex and age at diagnosis and height, weight, body mass index (BMI), height standard deviation score (SDS), weight SDS and BMI SDS levels were recorded. Ophthalmological examination, presence of skeletal involvements, adenoidal hypertrophy, hearing loss, organomegaly, pulmonary and cardiovascular complications, and surgical histories were noted. We collected the biochemical data to determine the function of the organs, GALNS enzyme levels, urine GAG and urine KS levels. Available radiological information also included. Six-minute walk test results were collected from the patients' records. Descriptive values of demographic and clinical parameters were analyzed.

Results

Mean age of the patients was 14.9±7.05 years (range; 5.5-36). Mean age at diagnosis was 7.3±6.2 years (range; 6 months-31 years). Female to male ratio was 13:12. Two patients were diagnosed at 1 to 3 years of age, 6 patients were diagnosed at 3 to 5 years of age, 13 patients were diagnosed at 5 to 10 years of age, and four of the patients were diagnosed at age more than 10 (Figure 1). Mean follow-up time was 7.4±3.5 years (range; 6 months-17 years). Failure to thrive was detected in all patients at the time of diagnosis accept one patient who was diagnosed at the age of 6 months; she had no clinical signs, and detection of the enzyme level was performed because she had a brother with MPS IVA. When she was one year old, she had the skeletal manifestation, short trunk, and kyphosis and the other systems involvement appeared during follow-up. Mean height SDS was -8.36±2.88 (range; -13.5 to -3.54), mean weight SDS was -3.4±1.1 (range; from -5.5 to -1.8) and mean BMI SDS was 0.9±1.03 (range; -0.96 to 3.04). Birth weights

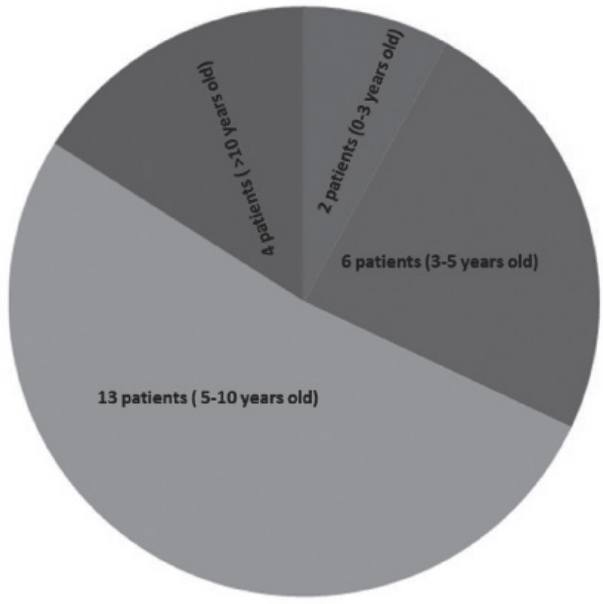

Figure 1. The ages of the patients at diagnosis

and heights of the patients were in normal range. All patients had urine GAG levels and enzyme activity of GALNS. Urine KS levels were available in 10 patients. Multiple sulphatase deficiency was ruled out for all patients. All patients had low enzyme activities of GALNS. Urine KS levels were increased in all 10 patients. The clinical and demographical details of the patients were summarized in Table I. All patients had skeletal manifestation including short trunk dwarfism, odontoid hypoplasia, pectus carinatum, kyphosis, gibbous, scoliosis, genu valgum, coxa valga, and hypermobile joint. X-ray analysis demonstrated "dysostosis multiplex" (DM) in all patients. Progression of anterior beaking, progressive kyphosis, platyspondyly and irregularities of the vertebral bodies were detected in all patients. The other clinical findings which were detected are corneal clouding, hearing loss, organomegaly, adenoid hypertrophy, cardiac valve disease and ventricular dysfunction. Clinical findings of the patients were detailed in Figure 2. Ten patients had recurrent pulmonary infections. Mild mental retardation was detected in 9 (92%) patients. Twenty-three patients had corneal clouding, one of them had corneal opacities. One had the plan of corneal transplantation.

Twelve patients had cardiac valve disease. Seven of them had mitral valve insufficiency; two of them had aortic valve insufficiency. Two of them had both aorta and mitral insufficiency. One patient had left ventricular dysfunction and treated with medical therapy. The others had no medical therapy due to cardiac involvement accept recommendation endocarditis prophylaxis. Complete blood count, biochemical analysis for liver and renal functions were in normal range. Three patients had vitamin D insufficiency. Spinal MR investigation was performed in 13 of patients. All had foramen magnum stenosis, intervertebral disc protrusion,

Table I. Summay of demographical and clinical features of the patients

Patient no	Gender	Age/ age at diagnosis (years)	Follow-up period (years)	Height SDS	Weight SDS	BMI SDS	Enzyme levels pmol/mg/h (400-2000) *nmol/ mg/17 h (45-249)	Clinical findings
1	F	16/6	10	-10.5	-3.47	2.76	3*	Coarse face, short stature, hepatomegaly, hearing loss, corneal clouding
2	F	14/7	7.5	-6.5	-3.5	0.14	13	Coarse face, short stature, corneal clouding
3	F	19/6	13	-5.06	-2.24	1.05	13	Coarse face, short stature, corneal clouding, adenoid hypertrophy
4	M	21/7	7	-11.5	-4.50	1.5	1*	Coarse face, short stature hepatomegaly, corneal clouding
5	F	15/10	9	-9.78	-5.07	0.08	2.8	Coarse face, short stature, hearing loss, corneal clouding adenoid hypertrophy
6	F	10/7	9	-5.39	-2.24	0.58	20	Coarse face, short stature, hepatomegaly, corneal clouding, mild retardation
7	M	8/3.5	7	-5.39	-2.27	1.02	0*	Coarse face, short stature, hearing loss, corneal clouding, mild retardation
8	M	21/1	9	-11.9	-5.26	0.01	2*	Coarse face, short stature, hepatomegaly, hearing loss, corneal clouding, adenoid hypertrophy
9	F	11/5.5	5.9	-8.58	-3.52	0.11	27.5	Coarse face, short stature, hearing loss, corneal clouding, mild retardation
10	F	24.5/2	17	-11.7	-4.83	2.10	2.2*	Coarse face, short stature, hearing loss, corneal clouding, adenoid hypertrophy
11	F	13.5/4.5	9	-9.61	-4.40	0.93	0.15*	Coarse face, short stature, hearing loss, mild retardation, adenoid hypertrophy
12	M	14/7	11	-6.6	-1.81	2.86	73	Coarse face, short stature, hearing loss, corneal clouding, mild retardation, adenoid hypertrophy
13	M	8/3.9	5.5	-5.79	-2.05	2.02	10	Coarse face, short stature, hearing loss, corneal clouding, mild retardation, adenoid hypertrophy
14	F	6/0.5	6	3.54	-2.05	0.11	35.2	Coarse face, short stature, corneal clouding
15	M	11/3	5.5	-7.32	-2.33	2.07	12.8	Coarse face, short stature, hearing loss, adenoid hypertrophy
16	F	12/7.3	5	-8.96	-3.50	0.91	35	Coarse face, short stature, hepatomegaly, hearing loss, corneal clouding, adenoid hypertrophy
17	F	8/3.5	5	-6.08	-2.41	0.76	40.9	Coarse face, short stature, corneal clouding
18	M	21/12	7	-12.46	-5.57	0.96	0*	Coarse face, short stature, hepatomegaly, hearing loss, corneal clouding
19	F	9.5/6	4	-6.94	-3.30	0.26	14	Coarse face, short stature, corneal clouding, mild retardation
20	F	36/31	4.5	-12.10	-4.69	0.4	0	Coarse face, short stature, hepatomegaly, hearing loss, corneal clouding
21	M	12/6	1	-6.01	-2.61	0.85	0	Coarse face, short stature hepatomegaly, corneal clouding
22	M	5.5/5	0.3	-4.5	-2.69	0.74	0	Coarse face, short stature, hepatomegaly, corneal clouding
23	M	25/15	10	-13.5	-4.90	0.01	16	Coarse face, short stature, hearing loss, corneal clouding, mild retardation, adenoid hypertrophy
24	M	18/8	10	-11	-4.20	0.84	34	Coarse face, short stature, hearing loss, corneal clouding
25	M	15/6	8	-8.30	-2.50	3.04	34	Coarse face, short stature, corneal clouding, mild retardation

BMI: Body mass index, SDS: Standard deviation score, F: Female, M: Male

loss of vertebral corpus height, malformations of vertebral bodies, platyspondyly, peri odontoid tissue, and ligaments thickening. Four patients had cervical myelopathy. Three patients had spinal cord compression and decompressive surgery is planned. Six (24%) patients were unable to walk, four (16%) patients walk less than 200 m, 13 (56%) patients walk between 200-400 m and one (4%) patient walk between 400-800 m. Bone mineral density (BMD) results were available in 10 patients and 7 of them had osteoporosis. Mean lumbar vertebrae Z scores was -2.8±2.9 (range; -9.4-

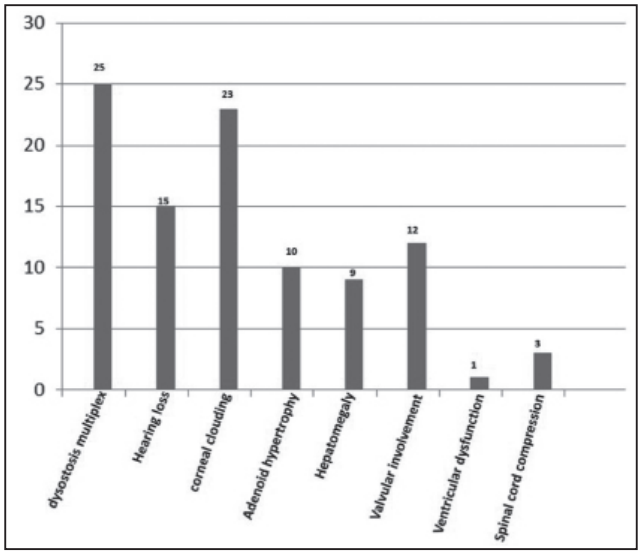

Figure 2. Clinical findings of the patients with mucopolysaccharidosis Type IVA

0.5) and mean neck of femur Z scores was -3.1±1.95 (range; -5.1-0.2). Seven patients had undergone adenoidectomy. Four patients had knee operation, two patients had hip operation, and 5 patients had the history of inguinal hernia repair. Mean age was 7.2±4.4 years (range; 3-16) for adenoidectomy, 10.5±2.3 years (range; 1.5-12) for orthopedic surgery, and 3.3±0.9 years (range; 2.5-5) for inguinal hernia repair.

Mean follow-up period is 7.4±3.5 years (range; 3 months-17 years). Four patients had not visit our clinical for more than 3 years. Three patients died during follow up. Two of them died due to the cardiopulmonary complications at the age 15 and 16. Other patient died due to respiratory insufficiency and infection while spinal cord decompression surgery was planned for him at the age of 17. Fourteen patients have been treated with enzyme elasulphase alpha. Mean period for ERT is 2±0.5 years (range; 3 months-2.9 years).

Discussion

The incidence of MPS IVA ranges among various populations from 1 in 76.000 live births in Northern Ireland to 1 in 640.000 live births in Western Australia (4-6). The higher incidence for Turkey is expected because of the high incidence of consanguinity marriages however we do not have a registration system for MPS IVA patients in our country. Over 70% of the patients with MPS IVA have initial clinical manifestations of skeletal features within the first 2-3 years of life (7). In our study, all patients had skeletal involvement. The common initial symptoms of our patients were short stature and gait disturbance and all have skeletal manifestations; as short trunk dwarfism, pectus carinatum, kyphosis, gibbous, scoliosis, genu valgum, coxa valga, hypermobile joint.

As reported by Montaño et al (7), musculoskeletal findings are a significant observation in patients with MPS IVA. An important component of the musculoskeletal manifestations in patients with MPS IVA is DM. All our patients showed the characteristics of DM. Progression of anterior beaking, progressive kyphosis, platyspondyly and irregularities of the vertebral bodies were detected. One patient was diagnosed at the age of 6 months, she had no clinical signs, and detection of the enzyme level was performed because she had a brother with MPS IVA. Although the clinical manifestations of skeletal systems started in early years of life, in our study 8 (32%) patients had the diagnosis of MPS IVA before the age 5. The one who was diagnosed at 6 months of age had skeletal manifestation when she was one years old. Diffuse corneal clouding is the most common ocular finding in MPS IVA. Photophobia is associated with stromal corneal clouding which is usually not severe. Retinopathy may occur (14). Couprie J et al. (14) investigated the ocular manifestations in patients with MPS IVA; the most common ocular manifestation was corneal opacification, astigmatism, and punctate cataract. Ophthalmological follow-up is recommended to detect potentially curable complications such as astigmatism or lens opacities (14). Glaucoma or ocular hypertension seems to be unusual in MPS IVA (15). In our study 23 (92%) patients had corneal clouding, none of them underwent eye surgery such as optical correction but one patient had corneal opacities. All forms of MPS, reduction in hearing can be attributed to multiple causes. Conductive hearing loss can be present due to recurrent upper respiratory tract infection. Most patients with MPS IVA have a mixed hearing loss; combination of a conductive element and sensorineural element (15). Hearing problems can become apparent by the end of the first decade of life (16). In our study 15 (25%) patients had hearing loss. Seven of the patients had hearing loss before the age of 10. Generally, dermatan sulphate deposition in the MPS syndromes results cardiac valve problems. However, KS and C6S are both found in normal cardiac valves and according to this, cardiac valve involvement found in MPS IVA patients (17). Mild mitral or aortic valvular disease is common while myocardial thickening, systemic and pulmonary hypertension, is rare. Bacterial endocarditis prophylaxis is advised for the MPS IVA patient with cardiac abnormalities (18). In our study 12 (48%) patients had cardiac valve disease. Similar to the literature most common valve insufficiency was in mitral or aortic valves. One patient had left ventricular dysfunction. The digestive system can be affected in patients with MPS IVA. According to the data of the International Morquio A registry which is conducted by the International Morquio Organization, some patients with MPS IVA had a hernia as their initial and current symptoms (7). In our study 5 of the patients had the history of inguinal hernia operation between the age of 2.5-5 years. Nelson et al. (19) reported hepatomegaly in their patients with MPS IVA, and was reported as an initial and current symptom in the International Morquio A Registry (7). We detected hepatomegaly in 9 patients. All our patients had normal liver functions. Suspicion of MPS IVA may be supported

by quantitative and/or qualitative testing of urinary GAG levels demonstrating increased levels and presence of KS, but diagnosis requires confirmation of GALNS deficiency in white blood cells or fibroblasts, or mutation analysis showing the presence of pathogenic mutations in both alleles (20,21). Enzyme activities of GALNS of all our patients were low. Urine KS analysis was available in 10 patients and all of them had elevated KS. We could not perform genetic analysis of our patients. Multiple sulphatase deficiency was ruled out in all patients. Endurance and functional capacity which is important outcome for MPS IVA patients, may be measured by the six-minute walk test (6MWT), which is simple and standardized measure of endurance (society AT, ATS statement: guidelines for the 6MWT A-11). In our study 6 (24%) patients were unable to walk, four (16%) patients walk less than 200 m, 13 (56%) patients walk between 200-400 m and one (4%) patient walk between 400 and 800 m. Maintenance of functional capacity and mobility are likely to improve quality of life of the patients with MPS IVA.

One of the most important and difficult aspect of this care is the management of airway. To prevent frequent respiratory infections, sleep apnea and breathing difficulty, the registry data indicated that tonsillectomy and adenoidectomy were performed at an early stage (the mean age is around 5 years) (7). In our study 9 patients had adenoid hypertrophy and 7 patients underwent adenoidectomy; unlike the literature mean age of adenoidectomy was around 10 years. The affected MPS IVA patients often require surgical procedures in neck, hip, knee, and leg regions in the first decade (7). Our findings were also similar to the literature. Orthopedic complications are the most critical issues for MPS IVA patients. Surgical interventions such as cervical fusion, decompression of the spinal cord, hip replacement are often required (7,22). None of our patients underwent surgery for spinal cord involvement. Although in three patients we planned spinal surgery, one patient died before the operation due to respiratory insufficiency and infectious complications and other two did not accept surgery. Kecskemethy et al. (23) showed that the lateral distal femur is the most feasible site to measure BMD in patients with MPS IVA. In our study, we have BMD results of 10 patients. We also think that because of the severe skeletal manifestations whole body mineral density should be performed. Davison et al. (24) demonstrated that central nervous system may not be entirely spared in MPS IVA as previously thought. Cognitive abnormalities and attention difficulties were reported. In our study 9 patients had mild retardation. We think that this finding may be result of social isolation and severe skeletal manifestation. Our study contains large number of MPS IVA patients from a single center and we presented our experience from a long survival period. In this study fourteen patients treated with enzyme elosulfase alpha. Mean period for ERT is 2±0.5 years (range; 3 months-2.9 years). Further studies with large number of patients may give us an opinion for the response to ERT in our region. Also after performing the gene analysis of the patients we may able to discuss the genotype and phenotype correlation of our patients.

Conclusion

MPS IVA is a severe progressive systemic skeletal disorder and is usually fatal in the second or third decade of life due to the complications. The disease has heterogeneous and progressive nature. Ideally, management should be centralized in major centers with access to all medical specialties. After the approval of the specific therapy with elosulfase alpha, early diagnosis of the patient became more important.

Ethics

Informed Consent: Consent form was filled out by all participants.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.C., H.Y., E.E., Concept: M.Ç., S.K.U., Design: M.Ç., S.K.U., Data Collection or Processing: E.C., H.Y., E.E., C.E., Analysis or Interpretation: E.C., M.Ç., S.K.U., Literature Search: E.C., H.Y., E.E., C.E., Writing: E.C., M.Ç., S.K.U.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Morquio L. Sur une forme de dystrophie osseuse familiale. Bull Soc Pediatr Paris 1929;27:145-52.
2. Dorfman A, Arbogast B, Matalon R. The enzymatic defects in Morquio and Maroteaux-Lamy syndrome. Adv Exp Med Biol 1976;68:261-76.
3. Glössl J, Kresse H. Impaired degradation of keratan sulphate by Morquio A fibroblasts. Biochem J 1982;203:335-8.
4. Meikle PJ, Hopwood JJ, Clague AE, Carey WF. Prevalence of lysosomal storage disorders, JAMA 1999;249-54.
5. J Nelson. Incidence of the mucopolysaccharidoses in Northern Ireland. Hum Genet 1997;355-8.
6. Nelson J, Crowhurst B, Carey L. Greed, Incidence of the mucopolysaccharidoses in Western Australia. Am J Med Genet 2003;310-3.
7. Montaño AM, Tomatsu S, Gottesman GS, Smith M, Orii T. International Morquio A Registry: clinical manifestation and natural course of Morquio A disease. J Inherit Metab Dis 2007;30:165-74.
8. Northover H, Cowie RA, Wraith JE. Mucopolysaccharidosis type IVA (Morquio syndrome): a clinical review. J Inherit Metab Dis 1996;19:357-65.
9. Tomatsu S, Okamura K, Taketani T, et al. Development and testing of new screening method for keratan sulfate in mucopolysaccharidosis IVA. Pediatr Res 2004;55:592-7.

10. Hendriksz CJ, Lavery C, Coker M, et al. Burden of disease in patients with Morquio A syndrome: results from an international patient-reported outcomes survey. Orphanet J Rare Dis 2014;9:32.
11. Masuno M, Tomatsu S, Nakashima Y, et al. Mucopolysaccharidosis IV A: assignment of the human N-acetylgalactosamine-6-sulfate sulfatase (GALNS) gene to chromosome 16q24. Genomics 1993;16:777-8.
12. Morris CP, Guo XH, Apostolou S, Hopwood JJ, Scott HS. Morquio A syndrome: cloning, sequence, and structure of the human N-acetylgalactosamine 6-sulfatase (GALNS) gene. Genomics 1994;22:652-4.
13. Hendriksz CJ. Elosulfase alfa (BMN 110) for the treatment of mucopolysaccharidosis IVA (Morquio A Syndrome). Expert Rev Clin Pharmacol 2016;9:1521-32.
14. Couprie J, Denis P, Guffon N, et al. Ocular manifestations in patients affected by Morquio syndrome (MPS IV). J Fr Ophtalmol 2010;33:617-22.
15. Hendriksz CJ, Al-Jawad M, Berger KI, et al. Clinical overview and treatment options for non-skeletal manifestations of mucopolysaccharidosis type IVA. J Inherit Metab Dis 2013;36:309-22.
16. Bredenkamp JK, Smith ME, Dudley JP, et al. Otolaryngologic manifestations of the mucopolysaccharidoses. Ann Otol Rhinol Laryngol 1992;101:472-8.
17. Dangel JH. Cardiovascular changes in children with mucopolysaccharide storage diseases and related disorders--clinical and echocardiographic findings in 64 patients. Eur J Pediatr 1998;157:534-8.
18. Tomatsu S, Montaño AM, Oikawa H, et al. Mucopolysaccharidosis type IVA (Morquio A disease): clinical review and current treatment. Curr Pharm Biotechnol 2011;12:931-45.
19. Nelson J, Thomas PS. Clinical findings in 12 patients with MPS IV A (Morquio's disease). Further evidence for heterogeneity. Part III: Odontoid dysplasia. Clin Genet 1988;33:126-30.
20. Wood TC, Harvey K, Beck M, et al. Diagnosing mucopolysaccharidosis IVA. J Inherit Metab Dis 2013;36:293-307.
21. Tomatsu AM, Montaño T, Nishioka MA, et al. Mutation and polymorphism spectrum of the GALNS gene in mucopolysaccharidosis IVA (Morquio A). Hum Mutat 2005;26:500-12.
22. Montaño AM, Tomatsu S, Brusius A, Smith M, Orii T. Growth charts for patients affected with Morquio A disease. Am J Med Genet 2008;15:1286-95.
23. Kecskemethy HH, Kubaski F, Harcke HT, Tomatsu S. Bone mineral density in MPS IV A (Morquio syndrome type A). Mol Genet Metab 2016;117:144-9.
24. Davison JE, Kearney S, Horton J, et al. Intellectual and neurological functioning in Morquio syndrome (MPS IVa). J Inherit Metab Dis 2013;36:323-8.

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Original Article

Clinical, Biochemical and Molecular Characteristics of Fifteen Patients with Mucopolysaccharidosis Type II in Western Turkey

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ABSTRACT

Aim: Mucopolysaccharidosis Type II (MPS II, Hunter syndrome, OMIM 309900) is a rare X-linked lysosomal storage disease due to a deficiency of the iduronate-2-sulfatase (IDS) enzyme, which is one of the degradative enzymes of mucopolysaccharides. The purpose of this study is to present the clinical, biochemical and molecular characteristics of fifteen patients with MPS II in western Turkey.

Materials and Methods: A retrospective study was carried out on fifteen patients with MPS II who were followed up by Ege University Faculty of Medicine, Unit of Pediatric Metabolic Diseases and Nutrition between October 2004 and September 2017.

Results: The age range of the patients enrolled in the study was between 11 months and 318 months at the time of diagnosis. The most common symptom was coarse face. On physical examination, all of the patients presented with coarse face, macrocephaly and organomegaly. Except for one patient, all other were severe phenotype. IDS activity was significantly decreased in all patients in whom enzyme analysis was performed. In this study, one novel mutation was described.

Conclusion: This is the first study on the clinical and molecular characterization of Turkish MPS II patients. The majority of the patients had neurologic involvement with different degrees of severity. The molecular analysis revealed one novel mutation.

Key words: Mucopolysaccharidosis Type II, Hunter syndrome, lysosomal storage disease, Turkey

Introduction

Mucopolysaccharidosis Type II (MPS II, Hunter syndrome, OMIM 309900) is one of the seven types of MPS I, II, III, IV, VI, VII, IX. This is a rare X-linked lysosomal storage disease due to a deficiency of the iduronate-2-sulfatase (IDS) enzyme, which is first degradative enzyme of mucopolysaccharides [now preferentially termed glycosaminoglycans, (GAGs)] dermatan sulfate and heparan sulfate. GAGs are essential constituents of connective tissue including cornea, cartilage, cardiac valve and vessel walls (Table I) (1). Partially digested dermatan sulfate and heparan sulfate accumulates and leads to multisystemic alterations including the skeleton, internal organs and central nervous system. The clinical characteristics of MPS II are macrocephaly, coarse face, hepatosplenomegaly, hernia, stiff joints, recurrent upper airway infections or otitis media, hearing loss, cardiac valve disease and/or cardiomyopathy and neurologic impairment. The *IDS* gene is located on Xq28 and contains 9 exons. The gold standard for diagnosis of MPS II in a male proband is reduced levels of IDS enzyme activity in fibroblasts, plasma or white cells and then diagnosis can be confirmed by means of *IDS* gene analysis. On exceptional occasions, heterozygous females present symptoms of MPS II. X inactivation, which

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is inactivation of normal paternally inherited X chromosome and expression of the maternally inherited X chromosome, can be the explanation for this issue (2,3). The *IDS* gene has three categories of alteration; large gene deletions, rearrangements and small gene deletions. More than 550 different mutations have been identified in the *IDS* gene to date, according to the Human Genome Mutation Database (HGMD® Professional 2016.2, www.hgmd.cf.ac.uk). Most of these are point mutations. IDS gene analysis is beneficial in diagnosing patients with unusual phenotypes and identifying genotype-phenotype correlations and it is also the only reliable way to define female carriers of the disease, which is a crucial factor in family planning (4). MPS II has multisystemic manifestations with variety in both the age of disease onset and rate of progression. Non-specific signs and symptoms that are similar to common childhood diseases and a lack of disease awareness cause delayed diagnosis (5). Although corneal clouding is not a typical feature of MPS II, it should not be forgotten. Two forms of the disease have been described. The most common form is often labelled as "early progressive" and manifests primarily with a combination of progressive cognitive deterioration and other multisystemic involvement. This form usually leads to death by the end of the second decade. The more infrequent form is often labelled as "slowly progressive", without or with a minimally affected central nervous system (6). The disease onset for the severe form is 12-18 months of age and for the attenuated form is 2-4 years of age (7).

Two main therapies have been reported for MPS II patients: The first one is idursulfase as an enzyme replacement therapy (ERT) which is a recombinant form of human IDS. This was approved in 2006 in individuals with the slowly progressing form of the disease. ERT has been shown to improve somatic symptoms, but the results with regard to cognitive functioning have been poor as idursulfase does not cross the blood-brain barrier (8). Hematopoietic stem-cell transplantation (HSCT) is reported as the other therapy. To date, only a few studies have examined the long-term outcomes of HSCT in patients with MPS II (9,10). HSCT can preserve neurocognition when performed early in the course of the disease. The need for early identification makes Hunter disease a candidate for new-born screening (NBS). Additionally, if NBS becomes widespread for MPS II, much milder presentations will be identified (11). Since there are no studies in Turkey on MPS II patients, the objective of this study is to present the genetic and clinical characteristics of patients in Western Turkey with Hunter syndrome.

Materials and Methods

A retrospective study was carried out on 15 patients with MPS II (from 13 families) and who were followed up by the Ege University Faculty of Medicine, Unit of Pediatric Metabolic Diseases and Nutrition, between October 2004 and September 2017. The data of patients including demographic, clinical, biochemical, radiological and mutation analyses were collected from their medical records. Diagnosis of MPS II was carried out by a detection of decreased levels of IDS enzyme activity in fibroblasts, plasma or white cells or by molecular genetic analysis. None of the patients were screened at birth for MPS II.

Statistical Analysis

The quantitative patient characteristics are summarized by means and standard deviations (SD). The qualitative characteristics such as general appearance and organ complications are presented as a frequency distribution.

Results

We collected data from 15 patients belonging to 13 families followed up by the Ege University Faculty of Medicine, Unit of Pediatric Metabolic Diseases and Nutrition. There were four patients in this study from two separate families. All patients were male. The patients age was between 11 months and 318 months at the time of diagnosis with a mean of 62 months and a median of 52 months. The youngest age at the diagnosis was eleven months and the oldest was 318 months. At the time of the study, the mean age of the living patients was 88.1 (SD 53.8) months. According to the birth weight data, two patients were "macrosomic", which literally means "big bodied", (defined by the American College of Obstetricians and Gynaecologists, as birth-weight >4000 g or >4500 g irrespective of gestational age). Another two patients were large for gestational age (LGA) (defined as a weight, length or head circumference that lies above the 90th percentile for gestational age). The clinical characterizations of the patients are summarized in

Table I. Pathological glycosaminoglycans in different mucopolysaccharidosis

	Normal	Mucopolysaccharidosis							Typical clinical findings, affected organ systems
		I	II	III	IV	VI	VII	IX	
Chondroitin sulphate	+				(+)		+	++	
Dermatan sulphate		++	++			++	n-+		Skeleton + internal organs
Heparan sulphate		+	+	+			n-+		Intellectual disability
Keratan sulphate					+				Skeleton

++: Prominent feature, +: Often present, (+): Sometimes present

Table II. The height of the patients was variable depending on their age (between +1.88 SD and -7.5 SD); under five years, 5-10 years and >10 years, the mean heights were +0.07±1.8 SD, -2.48±2.05 SD and -3.84±2.58 SD respectively. Figure 1 presents the main symptoms of the study group; the most common symptom was coarse face (100%), and the others, in order of prevalence, were developmental delay (93%), joint stiffness (60%), recurrent upper airway infections (47%), hernia (33%), convulsion (27%) and recurrent ear infections (20%). Coarse face, macrocephaly and organomegaly were the main findings detected by physical examination (Figure

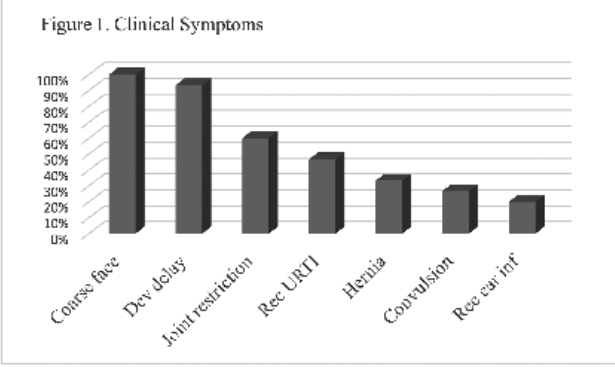

Figure 1. Clinical symptoms
Dev: Development, URTI: Upper respiratory tract infection, Rec: Recurrent, Inf: Infection

Table III. Iduronate-2 sulfatase activity

Patient	Enzyme activity	Normal range	Unit of measure	Sample type
H2	0	494-1113	nmol/mL/4 h	Plasma
H3	0.37	494-1113	nmol/mL	Plasma
H4	0.5	32-65	nmol/4 h/mg protein	Plasma
H6	0	35-110	nmol/4 h/mg protein	Fibroblast
H7	0.62	28.2±9.75	nmol/4 h/mg protein	Plasma
H8	0	494-1113	nmol/mL/4 h	Plasma
H8S	0	494-1113	nmol/mL/4 h	Plasma
H10	0	494-1113	nmol/mL/4 h	Plasma
H10S	0	494-1113	nmol/mL/4 h	Plasma
H13	0	494-1113	nmol/mL/4 h	Plasma
		Control		
H5	0	PC: 0.91 - NC: 0.74	nmol/mg/mL	Plasma
H9	0.06	NC1: 32.33 - NC2: 31.37	nmol/4 h/mg protein	Plasma
H11	0.71	NC1: 42.4 - NC2: 47.4	nmol/4 h/mg protein	Plasma

H: Hunter, NC: Normal control, PC: Positive control, S: Sibling

Table II. Clinical characteristics of mucopolysaccharidosis Type II patients

Patient	Last visit (m)	Starting therapy (m)	Diagnosis (m)	Current (m)	Valvulopathy	Cardiomyopathy	Hearing loss	Carpal tunnel	Hydrocephaly	Intellectual disability	SDS for height (last visit)
H1	140	No	12	Ex	+	+			+	+	
H2	127	No	53	Ex					+	+	-0.98
H3	353	354	318	Ex	+	-	+	+	-	-	-4.98
H4	160	95	64	Ex (159)	+	+	+	+	+	+	-5.36
H5	180	99	52	Ex (180)	+	+	+	+	-	+	-3.11
H6	179	82	24	Alive (178)	+	-	+	+	-	+	-7.50
H7	112	66	57	Ex (119)	+	-	+	+	-	+	-3.42
H8	80	No	68	Ex	+	+		+	+	+	-4.92
H8S	39	72	50	Alive (126)	+	+		-	-	+	-2.50
H9	134	53	52	Alive (133)	+	-	+	+	+	+	-1.10
H10	55	33	30	Alive (54)	-	-	-	-	-	+	-0.08
H10S	89	66	69	Alive (88)	+	-	+	+	+	+	-0.58
H11	37	24	20	Alive (36)	+	-	+	-	-	+	1.00
H12	21	13	11	Alive (21)	-	+	+	-	+	+	1.88
H13	70	1	62	Alive (69)					-	+	-1.00

1). Apart from H3, all of the patients presented neurologic involvement, mild to severe intellectual disability. H3 had no intellectual disability. Among follow up and living patients, five patients are able to walk without support and two patients are wheelchair-bound. Convulsion was noted in four patients 4/15 (26%). Cardiac involvement manifested at variable grades of valvulopathy (11/13-84%) and left ventricular hypertrophy (6/13-46%). In 11 patients with valvulopathy; mitral valve involvement was 90% (10/11), aortic valve involvement 63% and tricuspid valve involvement was 36% (4/11). On bone survey, dysostosis multiplex was noted in 12/12 patients. Hydrocephaly was noted in 7/15 patients on cranial imaging, and three of the seven patients with hydrocephaly had a shunt procedure. Carpal Tunnel syndrome was observed in eight patients on electromyography (8/12-66%). Three patients had broad Mongolian spot on physical examination. Three patients never received ERT and none of patients had no HSCT. ERT was started at the age of 83±76 months (limits: 11-319). Urinary GAG was elevated in all patients and increased dermatan sulfate and heparan sulfate was seen in GAG electrophoresis of 9 patients. IDS activity was significantly diminished in all patients in whom enzyme analysis was performed (Table III). Minor genetic defects were identified by molecular analysis (Table IV) in all patients in whom IDS gene analysis was performed, one of them was nonsense and the others were missense. One novel mutation was described.

Discussion

This is the first study on the clinical and gene mutation characterizations of Turkish MPS II patients. According to the birth weight data, two patients were macrosomic and another two patients were LGA. Previous studies have analysed birth parameters in patients with MPS II and mean birth weight has been reported to be slightly higher in MPS II patients than in those of the general population (15-19). Recently, a new large study from The Hunter Outcome Survey (HOS) also shows that birth weight is not associated with disease severity, in contrast to other previous studies (20). The age of admission or referral to a metabolic centre may be delayed. The need for early identification of mild presentations makes Hunter disease a candidate for NBS (11). The second most common symptom was developmental delay (93%), parallel to this data 14/15 patients had the severe form of the disease, which is consistent with recent studies (21-23). A total of 13/15 patients were assessed with echocardiography and cardiac involvement was detected in 12 of these patients. This data is compatible with the reports from HOS wherein the prevalence of cardiac presentation is high and valvular disease is the most common involvement (24-27). Three patients had broad Mongolian spot on physical examination. A clinical link between Mongolian spots and MPS II and other lysosomal storage diseases has already been reported in the literature (28-30). Mongolian spots may be one of the key factors in the early diagnosis of MPS II. IDS activity was significantly diminished in all patients in whom enzyme analysis was performed. Residual enzyme activity showed no predictive value (31).

Study Limitations

Limitations of the present study are that the enzyme levels were not measured for all of the patients and the molecular analysis of all patients were not taken into consideration.

Conclusions

This is the first study on the clinical and gene mutation characterization of Turkish MPS II patients. The clinical characteristics of MPS II in this case series were in agreement with what has been reported in that the age of diagnosis is much delayed despite an earlier onset of symptoms. Most of the patients had neurologic findings with different grades of severity. The molecular analysis revealed one novel mutation.

Ethics

Informed Consent: Informed consent was obtained.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.Y., E.C., H.O., F.Ö., Concept: S.K.U., Design: M.Ç., Data Collection and Processing: E.C., Analysis and Interpretation: H.Y., Literature Search: E.E., Writing: H.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declare that this study received no financial support.

Table IV. Mutation analysis of *IDS* gene

Patient	Mutation	Consequence	Location	Phenotype	Status	Reference
H3	c.322T>G	p.Y108D	Exon 3	Mild	Novel	-
H4	c.262C>T	p.R88C	Exon 3	Severe	Published	Rathmann et al., (12)
H6	c.262C>T	p.R88C	Exon 3	Severe	Published	Rathmann et al., (12)
H9	c.672G>A	p.G224E	Exon 5	Severe	Published	Karsten et al., (13)
H11	c.263G>A	p.R88H	Exon 3	Severe	Published	Rathmann et al., (12)
H12	c.162T>G	p.Y54X	Exon 2	Severe	Published	Mutesa et al., (14)

References

1. Guillén-Navarro E, Domingo-Jiménez MR, Alcalde-Martín C, et al. Clinical manifestations in female carriers of mucopolysaccharidosis type II: a Spanish cross-sectional study Orphanet J Rare Dis 2013;8:92.
2. Jurecka A, Krumina Z, Żuber Z, et al. Mucopolysaccharidosis type II in females and response to enzyme replacement therapy. Am J Med Genet A 2012;158:450-4.
3. Parenti G and Wraith EJ. (2014) The Mucopolysaccharidoses. In: Blau N, Duran M, Gibson KM, Dionisi-Vici C (eds) Physician's Guide to the Diagnosis,Treatment, and Follow-Up of Inherited Metabolic Diseases, Springer, Heidelberg, New York, Dordrecht, London, pp 449-64.
4. Scarpa M. Mucopolysaccharidosis Type II. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mefford HC, Stephens K, Amemiya A, Ledbetter N (eds) GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2017. 2007 Nov 6 [updated 2015 Mar 26].
5. Burton BK, Giugliani R. Diagnosing Hunter syndrome in pediatric practice: practical considerations and common pitfalls. Eur J Pediatr 2012;171:631-9.
6. Jones S, Wijburg F. Mucopolysaccharidoses, Oligosaccharidoses and Sialic Acid Disorders. In: Saudubray JM, van den Berghe G, Walter JH (eds) Inborn metabolic diseases, diagnosis and treatment, 6th edn. Springer, Heidelgerg, (2016) pp 577-88.
7. Wraith JE, Scarpa M, Beck M, et al. Mucopolysaccharidosis type II (Hunter syndrome): a clinical review and recommendations for treatment in the era of enzyme replacement therapy. Eur J Pediatr 2008;167:267-77. Epub 2007 Nov 23.
8. Muenzer J, Giugliani R, Scarpa M, et al. Clinical outcomes in idursulfase-treated patients with mucopolysaccharidosis type II: 3-year data from the hunter outcome survey (HOS). Orphanet J Rare Dis 2017;12:161.
9. Kubaski F, Yabe H, Suzuki Y, et al. Hematopoietic Stem Cell Transplantation for Patients with Mucopolysaccharidosis II. Biol Blood Marrow Transplant 2017;23:1795-803.
10. Barth AL, de Magalhães TSPC, Reis ABR, et al. Early hematopoietic stem cell transplantation in a patient with severe mucopolysaccharidosis II: A 7 years follow-up. Mol Genet Metab Rep 2017;12:62-68.
11. Ruijter GJ, Goudriaan DA, Boer AM, et al. Newbornscreening for hunter disease: a small scale feasibility study. JIMD Rep 2014;14:23-7.
12. Rathmann M, Bunge S, Beck M, et al. Mucopolysaccharidosis type II (Hunter syndrome): mutation "hot spots" in the iduronate-2-sulfatase gene. Am J Hum Genet 1996;59:1202-9.
13. Karsten S, Voskoboeva E, Tishkanina S, et al. Mutational spectrum of the iduronate-2-sulfatase (IDS) gene in 36 unrelated Russian MPS II patients. Hum Genet 1998;103:732-5.
14. Mutesa L, Muganga N, Lissens W, et al. Molecular analysis in two siblings African patients with severe form of Hunter Syndrome: identification of a novel (p.Y54X) nonsense mutation. J Trop Pediatr 2007;53:434-7.
15. Schwartz IV, Ribeiro MG, Mota JG, et al. A clinical study of 77 patients with mucopolysaccharidosis type II. Acta Paediatr 2007;96:63-70.
16. Rozdzynska A, Tylki-Szymanska A, Jurecka A, Cieslik J. Growth pattern and growth prediction of body height in children with mucopolysaccharidosis type II. Acta Paediatr 2011;100:456-60.
17. Patel P, Suzuki Y, Maeda M, et al. Growth charts for patients with Hunter syndrome. Mol Genet Metab Rep 2014;1:5-18.
18. Rozdzynska-Swiatkowska A, Jurecka A, Cieslik J, Tylki-Szymanska A. Growth patterns in children with mucopolysaccharidosis I and II. World J Pediatr 2015;11:226-31.
19. Rozdzynska-Swiatkowska A, Jurecka A, Zuber Z, Tylki-Szymanska A. Can macrosomia or large for gestational age be predictive of mucopolysaccharidosis type I, II and VI. Pediatr Neonatol 2016;57:181-7.
20. Bodamer O, Scarpa M, Hung C, Pulles T, Giugliani R. Birth weight in patients with mucopolysaccharidosis type II: data from the Hunter Outcome Survey (HOS). Mol Genet Metab Rep 2017;11:62-4.
21. Reboun M, Rybova J, Dobrovolny R, et al. X-chromosome inactivation analysis in different cell types and induced pluripotent stem cells elucidates the disease mechanism in a rare case of mucopolysaccharidosistype II in a female. Folia Biol (Praha) 2016;62:82-9.
22. Martin R, Beck M, Eng C, et al. Recognition and diagnosis of mucopolysaccharidosis II (Hunter syndrome). Pediatrics 2008;121:e377-86.
23. Beck M. Mucopolysaccharidosis type II (Hunter syndrome): clinical picture and treatment. Curr Pharm Biotechnol 2011;12:861-6.
24. Giugliani R, Villarreal ML, Valdez CA, et al. Guidelines for diagnosis and treatment of Hunter syndrome for clinicians in Latin America. Genet Mol Biol 2014;37:315-29.
25. Wraith J, Beck M, Giugliani R, et al. On Behalf Of The HOS Investigators. Initial report from the Hunter Outcome Survey. Genet Med 2008;10:508-16.
26. Young I, Harper P, Newcombe R, Archer I. A clinical and genetic study of Hunter's syndrome. 2 differences between mild and severe forms. J Med Genet 1982;19:408-11.
27. Arn P, Bruce IA, Wraith J, Travers H, Fallet S. Airway related symptoms and surgeries in patients with Mucopolysaccharidosis type I. Ann Otol Rhinol Laryngol 2015;124:198-205.
28. Ochiai T, Suzuki Y, Kato T, et al. Natural history of extensive Mongolian spots in MPS2 (Hunter syndrome): a survey among 52 Japanese patients. J Eur Acad Dermatol Venereol 2007;21:1082-5.
29. Musumeci ML, Lacarrubba F, Santagati C, Micali G. Multiple and superimposed Mongolian spots. BMJ Case Rep 2013;2013.
30. Gupta D, Thappa DM. Mongolian spots—how important are they? World J Clin Cases 2013;1:230-2.
31. Dvorakova L, Vlaskova H, Sarajlija A, et al. Genotype-phenotype correlation in 44 Czech, Slovak, Croatian and Serbian patients with mucopolysaccharidosis type II. Clin Genet 2017;91:787-96.

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Original Article

Clinical, Neuroimaging, and Genetic Features of the Patients with L-2-Hydroxyglutaric Aciduria

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ABSTRACT

Aim: L-2-hydroxyglutaric aciduria (L2HGA) is a rare autosomal recessive encephalopathy caused by mutations in the L-2-hydroxyglutarate dehydrogenase gene.

Materials and Methods: Here we discuss the clinical and molecular characteristics in patients with L2HGA.

Results: There were eight patients with L2HGA. Their median age was 16 (9.5-37) years. Five of them were female and three of them were male. The main symptoms of the patients were psychomotor retardation (8/8), cerebellar ataxia (5/8), extrapyramidal symptoms (7/8) and seizures (4/8). All patients had behavioral problems. Elevated urinary L-2-hydroxy (L-2-OH) glutaric acid was detected and the median level of urine L-2-OH glutaric acid at diagnosis was 146 (60-1460 nmol/mol creat). Characteristic magnetic resonance imaging findings including subcortical cerebral white matter abnormalities with T2 hyperintensities of the dentate nucleus, globus pallidus and putamen were detected. Two patients had homozygous R335X, two patients had homozygous R282Q, two patients had homozygous R302L and one patient had compound heterozygous P302L/A64T mutation in L-2-hydroxyglutarate dehydrogenase (L2HGDH) gene.

Conclusion: Because of the slow progression of the disease, the diagnosis of the patients is usually belated. L2HGA must be considered in the differential diagnosis based on clinical findings and specific findings in cranial magnetic resonance imaging. In our study, one of our patients has a novel mutation.

Keywords: L-2-hydroxyglutaric aciduria, ataxia, epilepsia, psychomotor retardation

Introduction

L-2-Hydroxyglutaric aciduria (L2HGA) is a very rare inherited metabolic disease with autosomal recessive inheritance [Online Mendelian Inheritance in Man (OMIM) #236792]. Since its first description in 1980 by Duran et al., (1) many additional cases of various ethnical backgrounds have been reported. Affected patients have slowly progressive deterioration with cerebellar ataxia, mild or moderate mental retardation, and extrapyramidal and pyramidal symptoms, and seizures and variable macrocephaly (2). L2HGA is characterized by elevated levels of L-2-hydroxyglutaric acid (L2HG) in urine, cerebrospinal fluid (CSF), and, to a lesser extent, plasma. Increased levels of L2HG are pathognomic for L2HGA (1,3). Neuroimaging findings generally show subcortical leukoencephalopathy, cerebellar atrophy and changes in dentate nuclei and putamen (4). L-2-hydroxyglutarate (Figure 1) in nature suggests that it is endogenously produced in humans. This result is due to the findings that the excretion of L-2-hydroxyglutarate in patients with L2HGA is little affected by the diet and that L-2-hydroxyglutarate accumulates in cultured cells deficient in L-2-hydroxyglutarate dehydrogenase (*L2HGDH*) (5). *L2HGDH* belongs to a large family of flavin adenine dinucleotide (FAD)-

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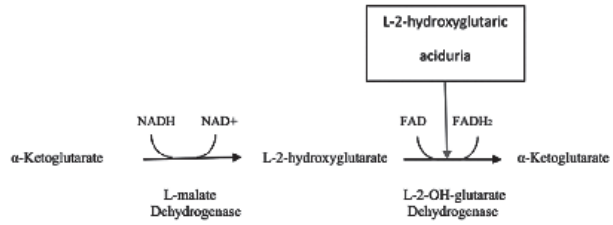

Figure 1. Oxidation of L-2-hydroxyglutaric acid to 2-ketoglutaric acid
FAD: Flavin adenine dinucleotide, L-2-OH: L-2-hydroxy, NADH: Nicotinamide adenine dinucleotide

linked dehydrogenases and oxidases. L-2-hydroxyglutarate is formed from alpha-ketoglutarate (metabolite in the tricarboxylic acid cycle) by the side activity of the mitochondrial L-malate dehydrogenase. L-2-hydroxyglutarate accumulation is toxic to the human brain, causing a leukoencephalopathy and increasing the sensibility to develop tumours (6). Episodes of acute metabolic decompensation do not occur, and brain damage is not related to acidosis in L2HGA patients. It's a difference from other forms of organic acidurias (7). The disease-causing gene, *L2HGDH* gene (*L2HGDH*), and its first pathogenic mutations were identified in 2004 (8,9). Treatment of L2HGA is under investigation; case reports have described positive effects of FAD sodium in combination with levocarnitine chloride in one patient (10) and riboflavin, a precursor of FAD, in another patient (11). The present study describes the clinical presentation and mutation analysis and follow up findings of our patients with L2HGA at Ege University Pediatric Metabolism and Nutrition Unit.

Materials and Methods

Eight patients who were diagnosed with L2HGA between the years of 2004-2016 were included in this study. Patients' demographical features; age, sex and age at diagnosis, consanguinity; clinical findings (such as; psychomotor retardation, loss of skills, extrapyramidal symptoms, seizures, behavioural problems) and head circumference (the presence of macrocephaly) were recorded. Urine 2-hydroxy (2-OH) glutaric acid levels at the diagnosis and L2HGDH gene analysis were analysed retrospectively. Radiological features were retrospectively included to the analysis.

Results

Median age of the patients was 17 (9.5-37) years and median age at diagnosis was 8 years (2-25 years). Female/male ratio of the patients was 5/3. Patient 1, 2 and Patient 6,7 were sibling. The main symptoms of the patients were psychomotor retardation (8/8), cerebellar ataxia (5/8), extrapyramidal symptoms (7/8), and seizures (4/8). All patients have behavioural problems. Five parents had consanguinity marriage. Five patients' head circumferences were known and none of them had macrocephaly. Clinical and demographical findings of the patients were detailed in Table I. Patient 6 diagnosed at the age of 25 years. Speech difficulties developed at the earlier ages, walking difficulties and seizures developed at the age of 25 years. Her sister with the same diagnosis (follow up at different clinic) had speech delay at the early childhood and learning difficulties at school age and tremor at the age of 15 years. All patients could walk without support before they lost of walking ability. Three patients' unsupported walking ages were not applicable. Patient 3 had psychomotor retardation and behavioural problems. He had milder clinic symptoms. Elevated urinary 2-OH glutaric acid was detected and median level of the urine 2-OH glutaric acid at the diagnosis was 146 (60-1460 nmol/mol creat). Two patients have homozygous R335X, two homozygous R282Q, two homozygous R302L, and one compound heterozygous P302L/A64T mutation in *L2HGDH* gene. One of the patient's *L2HGDH* gene analysis was not applicable. Detail diagnostic laboratory findings of the patients were given in Table II. Characteristic magnetic resonance imaging (MRI) findings including subcortical cerebral white matter (WM) abnormalities with T2 hyperintensities of the dentate nucleus, globus pallidus, putamen was detected. WM hyperintensities were detected in all patients. Dentate nucleus hyperintensities were detected in 3 patients, basal ganglion hyperintensities were detected in 5 patients and cystic encephalomalasia in one patient. Cranial MRI findings of the patient were detailed in Table III. In patient 2 cranial MRI shows WM involvement, cerebral atrophy and spongiform changes (Figure 2). In patient 3, cranial MRI reveals bilateral globus pallidus and caudate nucleus involvements (Figure 3).

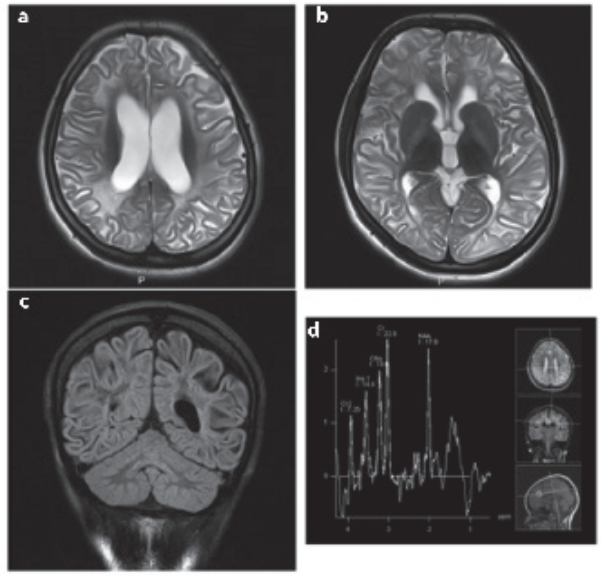

Figure 2. Axial T2 (a, b), coronal flair images, c) showing diffuse white matter hyperintentisities. Cerebral cortex and basal ganglions are spared. Multivoxel magnetic resonance spectroscopy images, d) showing reduced N-acetyl-aspartate level at the level of affected frontal white matter

Discussion

L2HGA is a very rare inherited metabolic disease with autosomal recessive inheritance (OMIM #236792). Affected individuals only have neurological manifestations, including mild to moderate psychomotor retardation, cerebellar ataxia, variable macrocephaly, and epilepsy (7,12-14). In our study similar to the literature the main symptoms of the patients were psychomotor retardation, cerebellar ataxia, extrapyramidal symptoms, and seizures. In the literature large studies demonstrated that during the disease course the main clinical findings were developmental delay (93%), cerebellar ataxia (82%), epilepsy (72%), and macrocephaly (48%) (2). It is interesting that in our study the available head circumferences were all normal. We couldn't get an information about the head circumferences of the 3 patients. In the earlier stages of the disease hypotonia was most prevalent and spasticity in the latter stages of disease in their cohort. Neurological decompensation (e.g., loss of skills and the development of speech deficits) was also present in a quarter of the patients (15). In our study loss of the skills were detected in 5 patients and all of them developed speech deficits. Also, all patients had behavioural problems. The clinical symptoms usually recognise during infancy or childhood. The cases in the literature reported as adult onset were diagnosed as adult onset but, in retrospect, the patients had symptoms during childhood (2). Patient 6 diagnosed at the age of 25 years. Speech difficulties developed at the earlier ages, walking difficulties and seizures developed at the age of 25 years. Her sister with the same diagnosis had speech delay at the early childhood and learning difficulties at school age and tremor at the age of 15 years. Mild symptoms may explain the late diagnosis ages. Seizures manifest in most of the patients (7). In our study 4 of the patients had the history of seizures. Patient 1 had the history of febrile seizures between the age of 3-4 years.

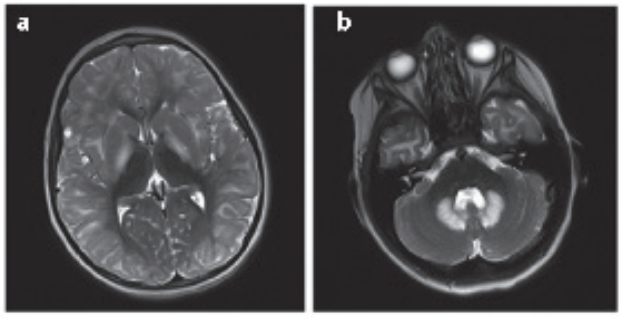

Figure 3. Axial T2 images showing hyperintentisities; a) bilateral globus pallidus, b) bilateral dentate nucleus

Table I. Demographical and clinical findings of the patients with L-2-hydroxyglutaric aciduria

Patient no	1[a]	2[a]	3	4	5	6[b]	7[b]	8
Age (year)	19	11	9.5	14	32	37	25	13
Age at diagnosis (year)	6	6	7	2	16	25	12	9
Gender	Female	Female	Male	Female	Female	Male	Female	Male
Consanguinity	+	+	+	-	+	+	+	+
Head circumferences	55.5 cm (50-98p)	52.5 cm (50-98p)	52 cm (50-98p)	55 cm (50-98p)	NA	NA	NA	55 cm (50-98p)
Psychomotor retardation	-	-	+	+	+	+	+	+
Unsupported walking age (months)	13	17	24	36	NA	NA	18	12
Loss of skills	+	+	-	+	-	+	+	-
Behavioural problems	+	+	+	+	+	+	+	+
Seizures	+	-	-	-	-	+	+	+
Cerebellar ataxia	+	+	-	+	+	+	-	-
Extrapyramidal symptoms	+	+	-	+	+	+	+	+

NA: Not applicable, [a]sibling, [b]sibling

Table II. Diagnostic laboratory findings of the patients with L-2-hydroxyglutaric aciduria

Patient no	1	2	3	4	5	6	7	8
Urine 2-OH glutaric acid (mmol/mol/ creat)	150	250	308	143	78	76	1460	135
L2HGDH gene mutations	R335X (CGA>TGA) homozygous	R335X (CGA>TGA) homozygous	p.P302L (c905C>T) /p.A64T (c.1906G>A) compound heterozygote	p.P302L (c905 C>T) homozygous	R282Q c.1003C>T homozygous	R282Q homozygous	NA	p.P302L (c905 C>T) homozygous

NA: Not applicable, *L2HGDH:* L-2-hydroxyglutarate dehydrogenase, 2-OH: 2-hydroxy

Table III. Cranial magnetic resonance imaging findings of the patients with L-2-hydroxyglutaric aciduria

Patient no	Cranial MRI findings
1	Bilateral cerebral atrophy, white matter T2 hyperintensities MR-S decreased NAA and choline peak
2	Bilateral cerebral atrophy, white matter T2 hyperintensities
	Bilateral dentate nucleus and capsula externa diffusion restriction
	Substantia nigra T2 hyperintensities
	MR-S decreased NAA and choline peak
3	Bilateral subcortical white matter hyperintensities
	MR-S decreased NAA and choline peak
4	Bilateral cerebral white matter T2 hyperintensities and diffusion restriction
	Bilateral putamen, bilateral caudate nucleus T2 hyperintensities and diffusion restriction
	Bilateral internal and external capsula hyperintensities
5	Bilateral cerebral white matter T2 hyperintensities
6	Bilateral cerebral hemisphere at basal ganglion levels cystic encephalomalasia
7	Bilateral subcortical white matter T2 hyperintensities Dentate nucleus T2 hyperintensities
8	Bilateral white matter T2 hyperintensities
	Basal ganglion T2 hyperintensities
	Bilateral nucleus dentate hyperintensities

MR-S: N-acetyl aspartate, MRI: Magnetic resonance imaging, NAA: N-acetyl-aspartate

The diagnosis is supported by increased levels of L-2-hydroxyglutarate acid in urine, plasma, and cerebro-spinal fluid (1). Some patients show increased levels of L-lysine and pipecolate in CSF suggesting a defect in alternative degradation pathway of lysine (16). We had not performed CSF analysis in our patients and plasma lysine levels were normal. We detected high 2-OH glutaric acid levels in all patients. Determination of L-2-OH isoform could not have performed. The diagnosis of the disease confirmed by *L2HGDH* gene analysis in 6 patients. *L2HGDH* gene analysis of the patient 7 was not applicable but she has high urine 2-OH glutaric acid levels and also she has the sibling with genetically diagnosed L2HGA (patient 6). Cranial MRI findings generally show subcortical leukoencephalopathy, cerebellar atrophy and changes in basal ganglion, in dentate nucleus (17). Steenweg et al. (17) investigated 56 patients with L2HGA and they found that with increasing disease duration, WM abnormalities and basal ganglia signal intensity abnormalities become more diffuse and cerebral WM atrophy arises. The few descriptions of histologic brain findings in L2HGA reported WM spongiosis, demyelination, and cystic degeneration, mostly in the subcortical regions (17). In our study all patients had WM changes on cranial MR. Three patients had bilateral dentate nucleus hyperintensities. Cranial MR investigation of patient 6 revealed cystic encephalomalasia. Işıkay (18) reported the case with L2HGA who had macrocephaly and cerebral multicystic lesions. Few data on proton MR spectroscopic (MR-S) changes are available. Anghileri et al. (19) detected 2 hydroxy glutarate peak in the WM of the three L2HGA patients. In our study decreased N-acetyl aspartate and choline peaks were detected on MR-S. Successful therapeutic trials have been reported in patients L2HGA. The patient described by Yilmaz (11) a 16 years old boy, had been treated with riboflavin for nearly 2 years and during treatment at 100 mg/day partial improvement in his cognitive and motor performances was observed and urinary secretion was decreased. Samuraki et al. (10) described 43 years old woman with L2HGA who was treated with FAD (30 mg/day) and carnitine. Significant improvement in her tremor and dystonia was detected and decreased urinary excretion of L2 hydroxy glutarate was detected (10). In our study half of the patients treated with riboflavin and due to the irregular follow up its difficult to give a comment on the treatment response. But we observed clinical stability. Two of our patients (siblings) have homozygous R335X (nonsense mutation) and 2 patients had homozygous p.P302L mutations. These mutations were reported in Turkish patients by Topcu et al. (8). Two patients had homozygous R282Q mutations which was previously reported by Samuraki et al. (10) Patient 3 had the p.P302L (c905 C>T)/p.A64T (c.1906G>A) compound heterozygote mutation. According to our knowledge p.A64T (c.1906G>A) had not been reported. Most patients reach adulthood. Increased incidence of brain tumours has been reported (20-22). A major difference between L2HGA and other dicarboxylic acidurias is that it is associated with a significant increase in the incidence of brain tumours. Six cases who developed brain tumour have been reported for a total of 100 known patients (6).

Conclusion

The symptoms of L2HGA are usually progressive. Some patients with milder clinical findings start having problems late in life. Early diagnosis of the patients may be useful because riboflavin treatment increases the activity of proteins in some patients. Regular follow up is also important because of the increased incidence of brain tumours.

Ethics

Informed Consent: Consent form was filled out by all participants.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.C., M.K., H.Y., E.E. Concept: E.C., M.Ç., S.K.U., Design: E.C., M.Ç., Data Collection or Processing: E.C., S.K.U., C.E., S.H., Analysis or Interpretation: E.K., H.O., F.Ö., Literature Search: E.C., S.K.U., Writing: E.C., M.Ç., S.K.U.

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References

1. Duran M, Kamerling JP, Bakker HD, van Gennip AH, Wadman SK. L-2-Hydroxyglutaric aciduria: an inborn error of metabolism? J Inherit Metab Dis 1980;3:109-12.
2. Steenweg ME, Jakobs C, Errami A, et al. An overview of L-2-hydroxyglutarate dehydrogenase gene (L2HGDH) variants: a genotype-phenotype study. Hum Mutat 2010;31:380-90.
3. Barth PG, Hoffmann GF, Jaeken JJ, et al. L-2-Hydroxyglutaric aciduria: a novel inherited neurometabolic disease. Ann Neurol 1992;32:66-71.
4. Steenweg ME, Salomons GS, Yapici Z, et al. L-2-Hydroxyglutaric aciduria: pattern of MR imaging abnormalities in 56 patients 1. Radiology 2009;251;856-65.
5. Struys EA, Verhoeven NM, Roos B, Jakobs C. Diseaserelated metabolites in culture medium of fibroblasts from patients with D-2-hydroxyglutaric aciduria, L-2-hydroxyglutaric aciduria, and combined D/L-2-hydroxyglutaric aciduria. Clin Chem 2003;49:1133-8.
6. Van Schaftingen E, Rzem R, Veiga-da-Cunha M. L:-2-Hydroxyglutaric aciduria, a disorder of metabolite repair. J Inherit Metab Dis 2009;32:135-42.
7. Topçu M, Aydin OF, Yalcinkaya C, et al. L-2-Hydroxyglutaric aciduria: a report of 29 patients. Turk J Pediatr 2005;47:1-7.
8. Topcu M, Jobard F, Halliez S, et al. L-2-Hydroxyglutaric aciduria: identification of a mutant gene C14orf160, localized on chromosome 14q22.1. Hum Mol Genet 2004;13:2803-11.
9. Rzem R, Veiga-da-Cunha M, et al. A gene encoding a putative FAD-dependent L-2-hydroxyglutarate dehydrogenase is mutated in L-2-hydroxyglutaric aciduria. Proc Natl Acad Sci U S A 2004;101:16849-54.
10. Samuraki M, Komai K, Hasegawa Y, et al. A successfully treated adult patient with L-2-hydroxyglutaric aciduria. Neurology 2008;70:1051-2.
11. Yilmaz K. Riboflavin treatment in a case with l-2-hydroxyglutaric aciduria. Eur J Paediatr Neurol 2009;13:57-60.
12. Barbot C, Fineza I, Diogo L, et al. L-2-Hydroxyglutaric aciduria: clinical, biochemical and magnetic resonance imaging in six Portuguese pediatric patients. Brain Dev 1997;19:268-73.
13. Barth PG, Hoffmann GF, Jaeken J, et al. L-2-Hydroxyglutaric acidaemia: clinical and biochemical findings in 12 patients and preliminary report on L-2-hydroxyacid dehydrogenase. J Inherit Metab Dis 1993;16:753-61.
14. Moroni I, D'Incerti L, Farina L, Rimoldi M, Uziel G. Clinical, biochemical andneuroradiological findings in L-2-hydroxyglutaric aciduria. Neurol Sci 2000;21:103-8.
15. Kranendijk M, Struys EA, Salomons GS, Van der Knaap MS, Jakobs C. Progress in understanding 2-hydroxyglutaric acidurias. J Inherit Metab Dis 2012;35:571-87.
16. de Klerk JB, Huijmans JG, Stroink H, et al. L-2-hydroxyglutaric aciduria: clinical heterogeneity versus biochemical homogeneity in a sibship. Neuropediatrics 1997;28:314-7.
17. Steenweg ME, Salomons GS, Yapici Z, et al. L-2-Hydroxyglutaric aciduria: pattern of MR imaging abnormalities in 56 patients. Radiology 2009;251:856-65.
18. Işıkay S. Cerebral multicystic lesions in a child with L-2 hydroxyglutaric aciduria: a rare disease and a rare association. Pediatr Neurol 2014;50:197-8.
19. Anghileri E, Bertolino N, Salsano E, et al. In-vivo brain H1-MR-Spectroscopy identification and quantification of 2-hydroxyglutarate in L-2-Hydroxyglutaric aciduria. Brain Res 2016;1648:506-11.
20. Aghili 1, Zahedi F, Rafiee E. Hydroxyglutaric aciduria and malignant brain tumor: a case report and literature review. J Neurooncol 2009;91:233-6.
21. Haliloglu G, Jobard F, Oguz KK, et al. L-2-hydroxyglutaric aciduria and brain tumors in children with mutations in the L2HGDH gene: neuroimaging findings. Neuropediatrics 2008;39:119-22.
22. Moroni I, Bugiani M, D'Incerti L, et al. L-2-hydroxyglutaric aciduria and brain malignant tumors: a predisposing condition? Neurology 2004;62:1882-4.

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Case Report

Dietary Management of a Patient with Both Maple Syrup Urine Disease and Type I Diabetes

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ABSTRACT

Maple Syrup Urine disease (MSUD) is caused by the deficiency of the branched chain 2-ketoacid dehydrogenase complex. Type I diabetes mellitus (TIDM) is a chronic illness characterized by the body's inability to produce insulin due to the autoimmune destruction of the beta cells in the pancreas. A case with both MSUD and TIDM has not been reported previously. In this study, we presented a patient with both MSUD and TIDM, dietary management and the follow up period. Dietary management could be difficult in the presence of two disorders affecting more than one macronutrient component such as both carbohydrate and protein. Our patient had good metabolic control in the follow-up period. Treatment was successful with intensive insulin, frequent feeding and a leucine restricted diet.

Keywords: Maple Syrup Urine disease, Type I diabetes mellitus, diet, children, metabolic control

Introduction

Maple Syrup Urine disease (MSUD) is caused by the deficiency of the branched chain 2-ketoacid dehydrogenase (BCKD) complex, the second common step in the catabolism of the three branched chain amino acids (BCAA), leucine, isoleucine and valine. Long term dietary treatment is aimed at reducing the accumulation of toxic metabolites, while at the same time maintaining normal physical development and nutritional status, and preventing catabolism. Type I diabetes mellitus (TIDM) is a chronic illness characterized by the body's inability to produce insulin due to the autoimmune destruction of the beta cells in the pancreas. Patients with TIDM need lifelong insulin therapy. One of the first steps in managing TIDM is dietary control. All patients on insulin should have a comprehensive diet plan, including a daily calorie intake prescription, recommendations for amounts of dietary carbohydrate, fat, and protein, and instructions on how to divide calories between meals and snacks. A case with both MSUD and TIDM has not been hitherto reported. Here, we present a patient with both MSUD and TIDM, and the dietary management of the patient.

Case Report

A four-year-old boy who was diagnosed with classical MSUD in the newborn period was admitted to our hospital with lethargy, polyuria and polydipsia. He had mild spasticity in the limbs and mild axial hypotonia as a sequel of a metabolic crisis attack in the newborn period. He had been found carrying homozygous c.757G>A; p.Ala253Thr mutation. At his last admission, his blood glucose level was 665 mg/dL. He had metabolic acidosis, and ketosis. Laboratory findings were: pH: 6.9, pO_2: 117 mmHg, pCO_2: 29.7 mmHg, HCO_3: 6.5, blood ketone level: 6.2 mmol/L. The hemoglobin A1c level was 11.2%. C-peptide level was low (0.486 ng/mL,

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normal range 0.9-7.1). Diabetes related autoantibodies were found positive and were as follows; islet cell antibody: 1:10 (n<1:10), anti-insuline antibody: 0.60% (n<0.40), anti-glutamic acid decarboxylase: 647.57 IU/mL (n<10). Leucine level was 451 µmol/L (n=55-164). Ketoacidosis was treated with intravenous regular insulin and fluid. Leucine free amino acid mixture was used for snacks until blood glucose levels were regulated. Following the recovery period, intensive insulin (four dosages a day) treatment was started. Three main meals and three snacks were commenced. The dietary plan of the patient consisted of 2 g/kg/day protein, 100 kcal/kg energy, and 30 mg/kg/day leucine. His dietary schema is shown in Figure 1. He is still going on with this diet along with thiamine treatment, and doing well. In the 1.5 year-period after the additional TIDM diagnosis, he has not experienced a metabolic crisis associated with TIDM or MSUD. He has only a mild global developmental delay.

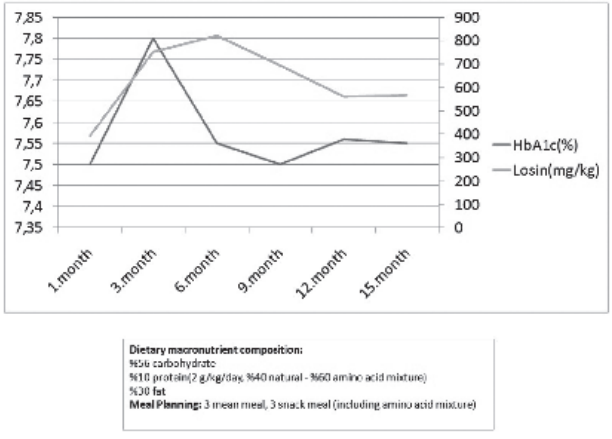

Figure 1. Clinical findings and dietary management of patient

Discussion

Here we presented a case with both MSUD and TIDM together with our dietary management approach. MSUD, also known as branched-chain ketoaciduria, is a disorder affecting the BCAA. MSUD occurs in approximately 1 in 86.800 to 185.000 live births (1,2). TIDM is a heterogeneous disorder characterized by the destruction of pancreatic beta cells, culminating in absolute insulin deficiency. Data from large epidemiologic studies worldwide indicate that the incidence of TIDM has been increasing by 2-5% worldwide and that the prevalence of TIDM is approximately 1 in 300 in the US by 18 years of age (3). Research on risk factors for TIDM is an active area of research to identify genetic and environmental triggers that could potentially be targeted for intervention. BCAA, which accumulates in Type II diabetes, contributes to insulin resistance. The activity of the BCKD complex, the rate-limiting enzyme in the BCAA catabolism, has clearly diminished. Therefore, diabetes contributes to the increase in BCAA. Lian et al. (4) demonstrated that mitochondrial phosphatase 2C (PP2Cm), which is associated with increased BCKD activity, reduced in hippocampal mice with Type II diabetes. Adiponectin (APN) is the new regulator of PP2Cm and BCAA. Targeting APN pharmacologically suggests that the catabolism of BCAA may be ameliorated. Our patient had TIDM, and diabetes related autoantibodies were demonstrated. Demonstration of the mechanism underlying the presence of two disorders together, MSUD and TIDM could be accepted as coincidence in this patient. Although he was treated with intensive insulin regimen, accumulation of BCAA may contribute to the increase of insulin in the follow-up period. Experimental treatments such as mitochondrial PP2Cm could be considered as a promising agent in the future. Exogenous (dietary) BCAA are major precursors for protein synthesis. Normally, they are also used as an alternative energy source when consumed in excess for anabolic needs or during endogenous muscle protein catabolism. The goals of medical nutrition therapy in MSUD are to rapidly reduce toxic metabolites by restricting dietary BCAA to amounts allowing individuals to achieve and maintain plasma BCAA amino acid concentrations within the targeted treatment ranges; reduce catabolism; promote anabolism; monitor nutritional status and alter intake to promote normal growth, development and health maintenance; evaluate thiamin responsiveness if the individual has residual BCKD activity; and supplement with thiamin if the individual is responsive (5). According to the Nutrition Management Guideline published by Frazier et al. (5), the recommended dietary requirements for children with MSUD were as follows; 35-65 mg/kg leucine, 20-30 mg/kg isoleucine, 30-50 mg/kg valine, 1.3-2.0 g/kg protein, and 50-120 kcal/kg energy. Our patient's dietary content was compatible with this guideline. Although median leucine level was slightly higher than the recommended levels, there was no metabolic disturbance and crisis other than the first metabolic crisis in the newborn period and during diabetic ketoacidosis attack. Significant advances have been made in the clinical care of TIDM. A number of therapeutic options for persons with TIDM currently exist, which include multiple daily injections of rapid acting insulin with meals combined with a daily basal insulin. In risky situations such as severe infections and occurrence of ketosis, high carbohydrate intake could be commenced with increased insulin doses.

In summary, dietary management could be difficult the presence of two disorders affecting more than one macronutrient component such as both carbohydrate and protein. Our patient had good metabolic control in the follow-up period. Successful treatment was achieved with intensive insulin, frequent feeding and a leucine restricted diet.

Ethics

Informed Consent: It was taken from the mother.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgicaland Medical Practices: M.G., N.K., Ö.Ü., S.A.U., Concept: M.G., N.K., Design: M.G., N.K., Ö.Ü., Data Collection

or Processing: N.K., S.A.U., Analysis or Interpretation: N.K., Literature Search: N.K., Writing: N.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Chuang DT, Shih VE. Maple syrup urine disease (branched-chain ketoaciduria). In: The metabolic and molecular bases of inherited disease, 8th ed, Scriver CR, Beaudet AL, Sly WS, Valle D (Eds), McGraw-Hill, New York 2001.p.1971.
2. Quental S, Vilarinho L, Martins E, et al. Incidence of maple syrup urine disease in Portugal. Mol Genet Metab 2010;100:385-7.
3. Maahs DM, West NA, Lawrence JM, et al. Chapter 1: Epidemiology of Type 1 Diabetes. Endocrinol Metab Clin North Am 2010;39:481-97.
4. Lian K, Du C, Liu Y, et al. Impaired adiponectin signaling contributes to disturbed catabolism of branched-chain amino acids in diabetic mice. Diabetes 2015;64:49-59.
5. Frazier DM, Allgeier C, Homer C, et al. Nutrition management guideline for maple syrup urine disease: an evidence- and consensus-based approach. Mol Genet Metab 2014;112:210-7.

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Case Report

"Double Hit" Homozygous Mutations for Two Different Rare Inborn Errors of Metabolism: A Burden for Countries with High Prevalences of Consangineous Marriages

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ABSTRACT

Inborn errors of metabolism comprise a broad range of genetic diseases of which most are inherited in an autosomal recessive manner. Although being rare, there is a significant increase in their rate especially in countries where consanguineous marriages are performed. Isovaleric aciduria is an organic aciduria characterized by abnormal leucine metabolism resulting from a deficiency in the enzyme isovaleryl-CoA dehydrogenase. Niemann-Pick disease Type C is a rare autosomal recessive inherited disorder involving the intracellular transport of endocytosed cholesterol with sequestration of unesterified cholesterol in lysosomes and late endosomes. Both of these disorders are rarely encountered inborn errors of metabolism. We report a case of a boy with marked jaundice and hepatosplenomegaly, who was later diagnosed as isovaleric aciduria and Niemann-Pick disease Type C concomitantly. The diagnoses were proven by genetic analyses. A novel mutation for Niemann-Pick Type C has also been defined in this case report.

Keywords: Inborn erors of metabolism, Niemann-Pick Type C, isovaleric aciduria, consanguinity

Introduction

Inborn errors of metabolism (IEM) are a group of genetically inherited disorders involving the effects of enzymes of the body on metabolism. Although being rare, there is a significant increase in their rate, especially in countries where consanguineous marriages are performed (1). We report a case of a 2-month old boy who was diagnosed as Isovaleric aciduria and Niemann-Pick disease Type C (NP-C) concomitantly.

Case report

Two-month old boy was admitted due to jaundice and marked hepatosplenomegaly. He was the 3rd child of consanguineous parents (first degree cousins). His prenatal, natal and family histories were uncomplicated. Jaundice had started when he was 5 days old and was still significant at the time of admission. Hepatosplenomegaly was detected in the outpatient clinic 8th during the routine second month examination and he was referred to our center for further evaluation. His body weight was 5.2 kg (25th percentile) and height was 60 cm (50th percentile). Physical examination revealed yellowing of the skin and eyes, hepatomegaly [4 cm palpable below the lower costal margin (LCM)] and splenomegaly (8 cm palpable below the LCM). Neurological examination showed normal findings. Complete blood count and peripheral blood smear showed normal results. Liver function tests revealed increased γ-glutamyl transferase (277

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U/L), alkaline phosphatase (473 U/L), conjugated bilirubine (5.25 mg/dL), serum glutamate pyruvate transaminase (SGPT) 64 U/L, serum glutamate oxaloacetate transaminase (SGOT) 264 U/L, with normal albumin and International Normalized Ratio (INR). Arterial blood gas analysis, serum lactate and ammonia were within normal limits. Abdominal ultrasonography showed hepatosplenomegaly. He was further evaluated by immunoglobulins, creatine kinase, amylase, thyroid function tests, alfa 1-antitripsine, ceruloplasmine, urine analysis and Benedict's test of urine which showed normal results. TORCH and hepatitis serology were negative. Chitotriosidase (165, normal range 0-120) and alpha feto protein (191.029 ng/mL, normal range 0-7) were elevated. Tandem mass spectrometric analysis using dried blood spots indicated an elevated isovaleryl carnitine level (4 µmol/L [not reported (NR) <0.6 µmol/L]. Free carnitine level was within normal limits. His urine organic acid profile showed a markedly increased concentration of isovalerylglycine [80 mmol/mol creatinine (Cr), normal value: 0], and a normal concentration of 3-hydroxyisovaleric acid (6.5 mmol/mol Cr; NR: 0.7-14.4 mmol/mol Cr). In respect to the results of the metabolic tests, genetic testing with Next Generation Sequencing (Miseq-Illumina) for the *IVD* gene was performed using genomic DNA which was isolated from his peripheral leukocytes due to a suspicion of isovaleric acidemia (IVA). A previously reported p.A314V (c.941C>T) homozygous mutation was detected (Figure 1). Although a diagnosis was reached, the patient's splenomegaly still could not be explained and further investigation was essential. With this aim, bone marrow biopsy was performed which revealed macrophages with abnormal cholesterol storage (Figure 2). Lysosomal enzyme studies from leukocytes were carried out for glucocerebrosidase, sphingomyelinase and acid lipase enzymes which were 5.93 nmol/h/mg protein (normal range 9.4±3.2), 5.13 nmol/h/mg protein (normal 7.73±3.08) and 150 nmol/h/mg protein (normal range 62.47±35.9) respectively. Since these results made the condition unlikely to be as a result of Gaucher, Niemann-Pick disease Type A-B and Wolman diseases, further studies were performed including Next Generation Sequencing

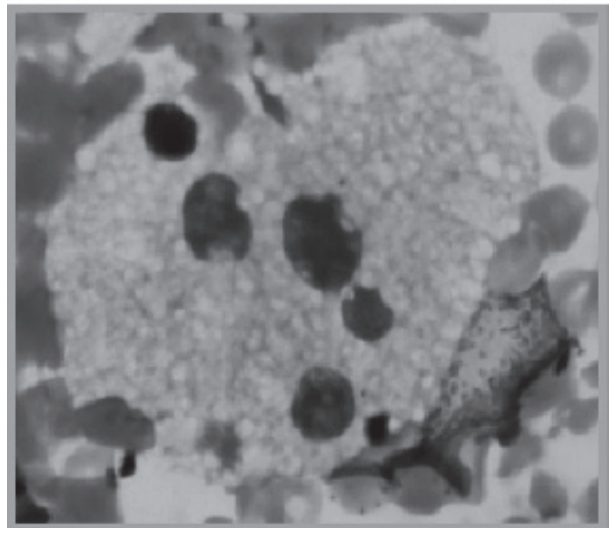

Figure 2. Bone marrow biopsy showing macrophages with abnormal cholesterol storage

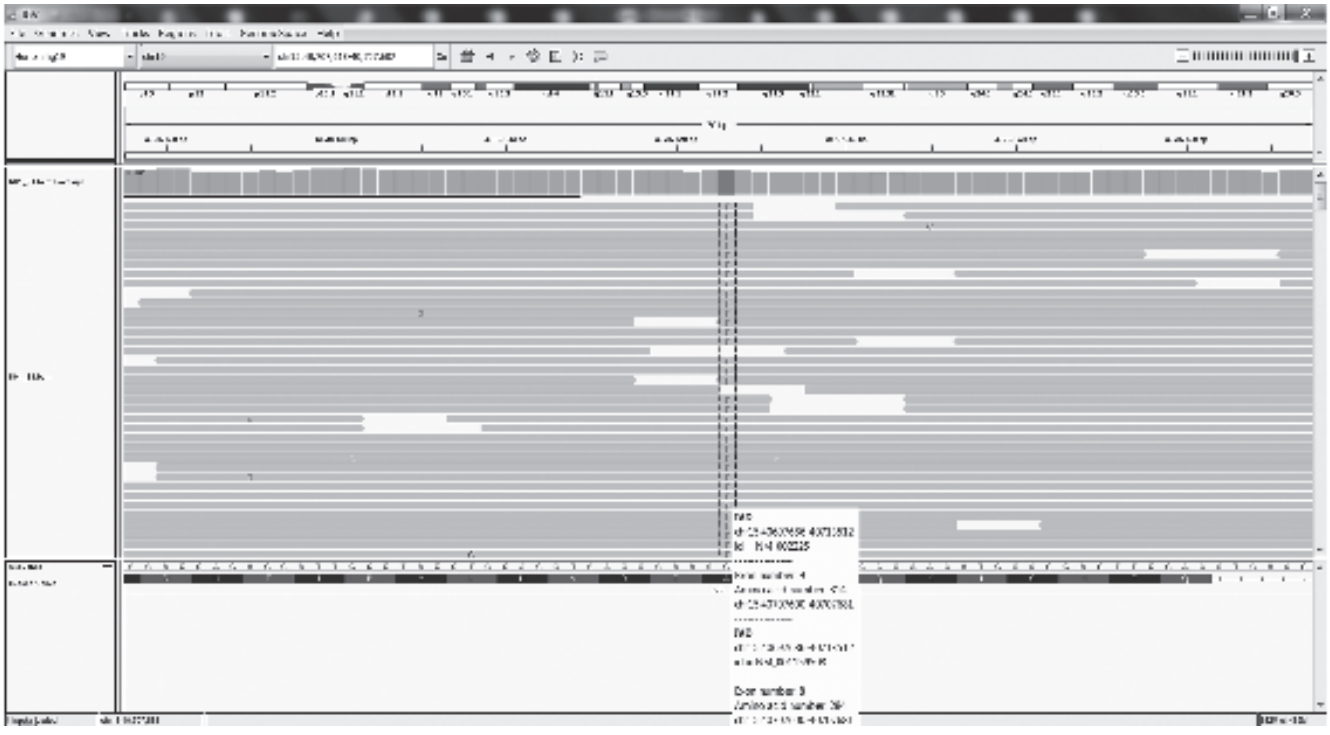

Figure 1. Sequence analysis for *IVD* gene

of *NPC1* gene, which was carried out on DNA isolated from leukocytes, for NP-C. A novel homozygous frameshift mutation in *NPC1* gene: p.P733Sfs*10 (c.2196_2197insT) was detected (Figure 3). Both of the boy's parents were shown to carry both mutations. The patient had remained in a stable condition on glycine supplementation and low-leucine diet. After the age of 1, mild psychomotor retardation, vertical supranuclear gaze palsy and mild spasticity were detected upon physical examination. Electroencephalogram showed irregular background activity with focal slowing. Miglustat therapy was initiated. His neurological condition remained stable after miglustat therapy. He died suddenly at home during an episode of aspiration pneumonia at the age of 2.

Discussion

IEM comprise a broad range of genetic diseases of which most are inherited in an autosomal recessive manner. Individual IEM are rare, most having an incidence of less than 1 per 100.000 births, increasing with the prevalence of consanguineous marriages (2). The incidence of two different IEM occurring concomitantly in a patient has been rarely reported in literature. Guy et al. (3) reported Fabry disease and aspartylglucosaminuria in a patient with consangineous parents. The authors emphasized consanguinity as a factor in increasing the risk of autosomal recessive disorders occurring concomitantly (3). Concolino et al. (4) reported phenylketonuria and Fabry disease to co-exist in a 3-year old boy with consaguineous parents. IVA is a type of organic aciduria characterized by an abnormal leucine metabolism resulting from the deficiency in the enzyme isovaleryl-CoA dehydrogenase. The incidence is estimated to be 1 in 62.500-250.000 (5). Currently, there are no published data about the incidence of IVA for Turkey. To date, more than 40 heterogeneous mutations in the *IVD* gene (Online Mendelian Inheritance in Man: 607036), located on chromosome 15q14-15 (http://www.hgmd.org/) have been reported. Missense and splicing mutations are the most common. The p.A314V (c.941C>T) homozygous mutation in the *IVD* gene of our patient is a common frameshift mutation and has been previously reported with 20% residual enzyme activity (6). Although it is mostly accepted to cause a mild, asymptomatic clinic with normal development, it is still uncertain whether individuals have a risk of clinical manifestation. While these individuals may have normal leucine homeostasis under physiological conditions, their risk of metabolic decompensation under stress conditions remains to be determined (5). Nasser et al. (7) showed that the A314V (Also reported as A282V) mutant enzyme had thermal instability, a finding that might play a role in the development of symptoms during times of illnes. In our patient, initially, a low leucine diet was introduced with a further plan of transforming into a normal protein diet in further follow-up, since the risk of acute decompensation of IVA could not be ruled out completely, as it has been reported in the literature. The low leucine was continued until he was one year old. None of his febrile attacks had been accompanied by metabolic decompensation during this period. NP-C is a rare disorder of intracellular transport of endocytosed cholesterol with sequestration of unesterified cholesterol in lysosomes

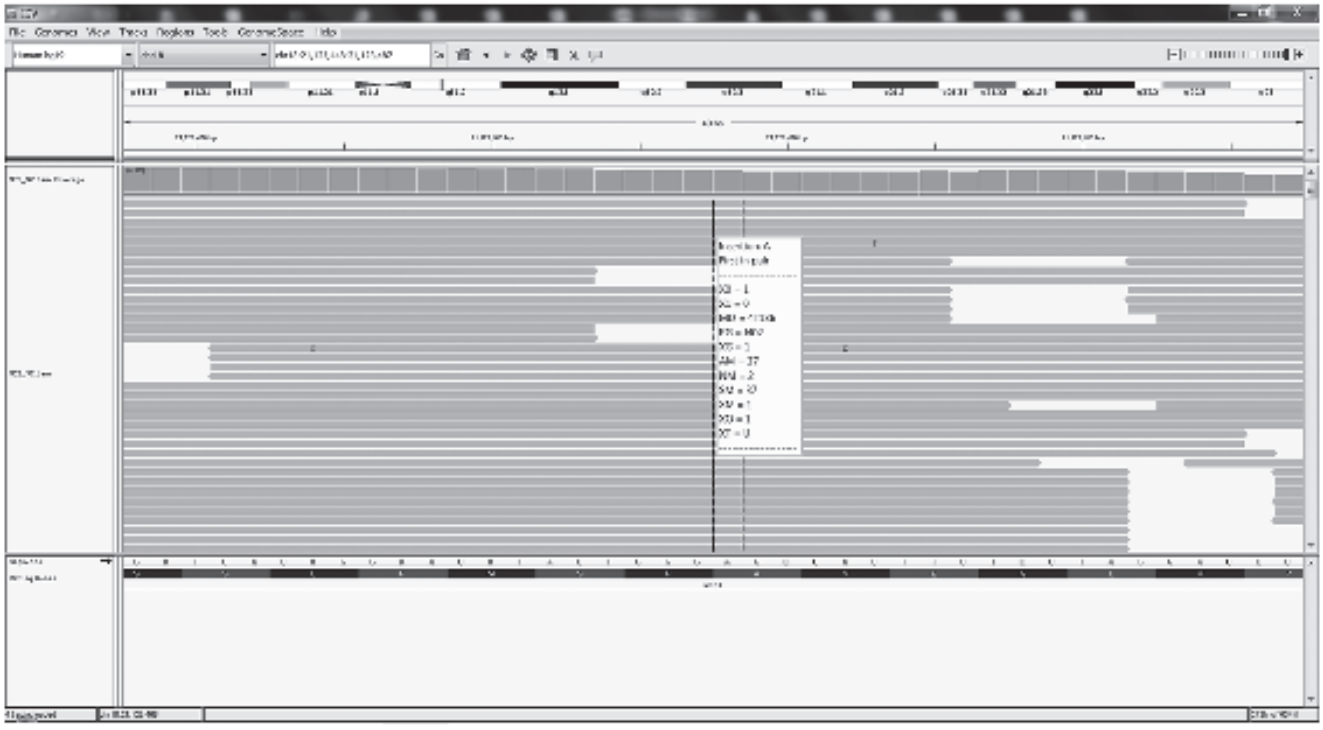

Figure 3. Sequence analysis for *NPC* gene

and late endosomes. Most of its patients (95%) have mutations in the *NPC1* gene (8). The prevalence in Western Europe has been estimated to be approximately 0.0008-0.0007% of live births. Again, there are no specific data for Turkey. To date, over 300 disease-causing mutations have been reported, most being missense mutations (9). Different clinical phenotypes of the disease can be categorized according to the patient's age at onset of signs. Perinatal presentation is characterized by a prolonged neonatal cholestatic icterus and progressive hepatosplenomegaly, appearing in the first days of life, usually associated with fetal hydrops or fetal ascites. Early-infantile type is characterized by hepatosplenomegaly with neurological findings occurring between 2-24 months. Prolonged neonatal cholestatic icterus associated with progressive hepatosplenomegaly is the most common sign with spontaneous resolution of the icterus by 2-4 months of age. Main neurological symptoms are ataxia, vertical supranuclear gaze palsy (which is considered highly characteristic), dysarthria, dysphagia, dystonia, seizures, cataplexy and progressive dementia (8-10). As reported in the literature, our patient's findings (prolonged jaundice and hepatosplenomegaly which showed up in the second month of life) suggest that he had the early-infantile type of the disease. Neurological signs including psychomotor delay, focal seizures and mild spasticity appeared after 12 months. The mutation of NPC1 gene described in this study p.P733Sfs*10 (c.2196_2197insT) is a novel frameshift mutation leading to a stop codon (http://www.hgmd.org/). As the result is a truncated protein, this mutation most probably causes severe damage to its function. Wild Type protein has 1278 amino acids, while this truncated protein has just 743 amino acids. The disease has been reported with proteins with larger amino acid content. The disease management may include drugs and supportive therapy. Miglustat (ZavescaW, Actelion Pharmaceuticals Ltd, Switzerland), is a reversible inhibitor of glucosylceramide synthase, that has been shown to be effective in the treatment of progressive neurological manifestations in particularly among those with Late-infantile or Juvenile-onset disease, and has been used in that indication in Europe since 2010. It can be initiated as soon as neurological conditions occur. New therapies are in development based on experimental data on substrate reduction therapies (10). In our patient, no seizures were observed after the initiation of miglustat. Although he remained stable, sudden cardiac arrest occured at home. The exact cause of death could not be identified and an autopsy was recommended which the family refused. Laboratory data which were obtained a few days before his death, in an outpatient clinic showed elevated acute phase reactants, and normal blood gas and ammonia levels. Unfortunately, test to identify his metabolic state regarding isovaleric aciduria could not be performed.

When the clinical picture in a patient cannot be explained by the presence of one rare metabolic disease, it is essential to investigate further for the presence of other IEM's and more attention should be given in individuals with consanguineous parents. We also would like to emphasize the importance of NBS in the early detection of rare metabolic diseases and more care should be given for the extension of NBS programmes, especially in developing countries. A novel mutation for NPC has also been described.

Ethics

Informed Consent: Has been obtained.
Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.O., L.T., S.C., G.B., A.H., Concept: A.O., L.T., S.C., G.B., A.H., Design: A.O., L.T., S.C., G.B., A.H., Data Collection or Processing: A.O., L.T., S.C., G.B., A.H., Analysis or Interpretation: A.O., L.T., S.C., G.B., A.H., Literature Search: A.O., L.T., S.C., G.B., A.H., Writing: A.O., L.T., S.C., G.B., A.H.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Bittles A. Consanguinity and its relevance to clinical genetics. Clin Genet 2001;60:89-98.
2. Applegarth DA, Toone JR, Lowry RB. Incidence of Inborn errors of metabolism in British Columbia. Pediatrics 2000;105:E10.
3. Guy R, Forsyth JM, Cooper A, Morton RE. Co-existence of lysosomal storage diseases in a consanguineous family. Child Care Health Dev 2001;27:173-81.
4. Concolino D, Rapsomaniki M, Disabella E, et al. Co-existence of Phenylketonuria and Fabry disease on a 3 year-old boy: case report. BMC Pediatr 2010;10:32. Published online 2010.
5. Ensenauer R, Vockley J, Willard JM, et al. A common mutation is associated with a mild, potentially asymptomatic phenotype in patients with isovaleric acidemia diagnosed by newborn screening. Am J Hum Genet 2004;75:1136-42. Epub 2004 Oct 14
6. Mohsen AW, Anderson BD, Volchenboum SL, et al. Characterization of molecular defects in isovaleryl-CoA dehydrogenase in patients with isovaleric acidemia. Biochemistry 1998;37:10325-35.
7. Nasser I, Mohsen AW, Jelesarov I, et al. Thermal unfolding of medium chain acyl-CoA dehydrogenase and iso(3)valeryl-CoA dehydrogenase: study of the effect of genetic defects on enzyme stability. Biochim Biophys Acta 2004;1690:22-32.
8. Vanier MT. Niemann-Pick disease type C. Orphanet J Rare Dis 2010;5:16.
9. Rodríguez-Pascau L, Toma C, Macías-Vidal J, et al. Characterisation of two deletions involving NPC1 and flanking genes in Niemann-Pick type C disease patients. Mol Genet Metab 2012;107:716-20.
10. Megías-Vericat JE, García-Robles A, Company-Albir MJ, et al. Early experience with compassionate use of 2 hydroxypropyl-beta-cyclodextrin for Niemann Picktype C disease: review of initial published cases. Neurol Sci 2017.

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Case Report

Siblings with Ethylmalonic Encephalopathy: Case Report

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ABSTRACT

Deficiency of mitochondrial sulfur dioxygenase (ETHE1) causes a rare inborn error of metabolism, ethylmalonic encephalopathy, which is characterized by early-onset encephalopathy, chronic hemorrhagic diarrhea, recurrent petechiae, orthostatic acrocyanosis, defective cytochrome C oxidase because of hydrogen sulfide accumulation and death in the first years of life. Biochemical hallmarks of the disease are high level of lactate, C4-C5-acylcarnitines in blood and markedly elevated urinary excretion of methylsuccinic and ethylmalonic acids. We report on two siblings who were admitted to a pediatric metabolic unit with acrocyanosis, chronic diarrhea and psychomotor retardation later diagnosed as ethylmalonic encephalopathy. Molecular analyses revealed a homozygous for p.R163Q (c.488 G>A) mutation in *ETHE1* gene.

Keywords: Acrocyanosis,developmental delay, ETHE1, ethylmalonic encephalopathy

Introduction

Ethylmalonic encephalopathy (EE) is an autosomal recessive metabolic disease caused by mutations in the mitochondrial sulfur dioxygenase (*ETHE1*) gene (1). The disease is characterized by an early onset of neurological degeneration, chronic hemorrhagic diarrhea, recurrent petechiae, orthostatic acrocyanosis and death in the first years of life (2-4). Biochemically, these patients have increased urinary excretion of ethylmalonic acid, along with 2-methylsuccinate, butyrylglycine and isovalerylglycine. Along with elevated plasma concentrations of C4 and C5 acylcarnitine species, the patients may experience recurrent or persistent bouts of lactic acidemia and may have cytochrome oxidase deficiency on muscle biopsy. Dysmorphic facial features include the presence of epicanthal folds and a broad nasal bridge. The onset of the disease is during the first months of life and children usually die within the first decade (5,6).We report on siblings with EE who were admitted to the pediatric metabolic unit with acrocyanosis, chronic diarrhea and psychomotor retardation.

Case Report

A 3 year 9 month old boy was born to consanguineous Turkish parents (first cousins) at term by normal vaginal delivery after an uneventful pregnancy. His growth parameters were as follows: weight 9800 g (<3p), length 82 cm (<3p), head circumference 48 cm (<3p). Their first child, a 6 year and 3 month old female, was born at 39 weeks, with a birth weight of 3600 g. She was firstly admitted to the pediatric nephrology department because of acrocyanosis (Figure 1). Serologic studies were negative for C-reactive protein, serum complement C3, C4, antinuclear antibody, anti-dsDNA and rheumatoid factor. She was admitted to the pediatric metabolic unit with orthostatic acrocyanosis, psychomotor delay and chronic diarrhea. Her growth parameters were as

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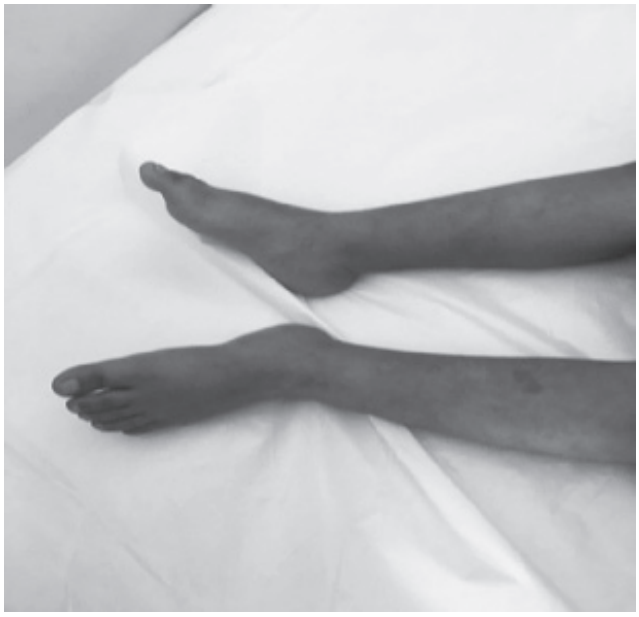

Figure 1. Ortostatic acrocyanosis of the boy with ethylmalonic encephalopathy

follows: weight 10 kg (<3p), length 85 cm (<3p) and head circumference 48.5 cm (<3p). Neurological examination of the siblings revealed axial hypotonia, spasticity of upper and lower extremities, brisk deep tendon reflexes and clonus. This clinical picture led to the suspicion of EE. The elder sister presented all the typical biochemical hallmarks of the disease including elevated lactate and butyrylcarnitine [C4: 2.89 µmol/L normal values (NV: 0.04-1.5)] in blood and elevated urinary excretion of ethylmalonic acid 93.63 µmol/mmol creatinine (NV: 1.7-14.6), isobutyrylglycine 1.05 µmol/mmol creatinine (NV: 0) and isovalerylglycine 4.9 µmol/mmol creatinine (NV: 0). The second child was clinically similar and had elevated plasma lactate levels on several occasions and urine organic acids consistently showed elevations of ethylmalonic acid [83 µmol/mmol creatinine (NV: 1.7-14.6)], methylsuccinate, isobutyrylglycine 2.62 µmol/mmol creatinine (NV: 0) and isovalerylglycine 11.95 µmol/mmol creatinine (NV: 0). Plasma acylcarnitine analyses showed elevations of short chain species of butyrylcarnitine and isovalerylcarnitine. A peripheral blood sample was collected from the patient after we obtained informed consent. The *ETHE1* gene was sequenced and they were both shown to be homozygous for the p.R163Q (c.488G>A) mutation while both of the parents were heterozygous for the same mutation. The p.R163Q (c.488G>A) mutation has been described previously in EE. On follow-up, combined treatment with oral metronidazole 30 mg/kg/day, N-acetylcysteine100 mg/kg/day, coenzyme Q10 5 mg/kg/day and riboflavin 100 mg/day were administered with clinical improvement (mild neurological improvement, partial control of diarrhea and disappearance of acrocyanosis).

Discussion

EE (EE; OMIM No. 602473) is a fatal infantile disease caused by an accumulation of sulfide, H_2S, a mitochondrial poison produced exogenously by the anaerobic enterobacterial flora and synthesized endogenously in various mammalian tissues (5). Failure to detoxify sulfide is due to the absence or malfunctioning of a mitochondrial sulfur dioxygenase, encoded by the *ETHE1* gene, which is mutated in EE, and characterized by ethylmalonic and methylsuccinic aciduria and lactic acidemia associated with neurodevelopmental delay and regression, pyramidal and extrapyramidal signs, vascular lesions determining episodes of acrocyanosis, recurrent petechiaea and chronic diarrhea (6-8).Our patients had acrocyanosis, chronic diarrhea and psychomotor delay. Acrocyanosis appears as a bluish discoloration of the skin and mucous membrane because of diminished oxyhemoglobin. It is caused by chronic vasospasm of small cutaneous arteries and arterioles along with compensatory dilatation in the capillaries. It can be both primary and secondary to psychiatric, neurologic, autoimmune, infective and metabolic causes. Orthostatic acrocyanosis is one of the principal signs of EE. Inherited metabolic disorders causing acrocyanosis also include fucosidosis, hyperoxaluria Type I, congenital disorder of glycosylation and mitochondrial disorders with onset in early infancy and multisystem involvement. Persistent anorexia, feeding difficulties, chronic vomiting, failure to thrive, frequent infections, generalized hypotonia and neurological findings in association with chronic diarrhea occur in a wide variety of inborn errors of metabolism. Mitochondrial Neurogastrointestinal Encephalopathy (MNGIE) syndrome, menkes disease, congenital disorder of glycosylation, hartnup disease and disorders of cobalamin and folate metabolism and transport are examples of metabolic disorders which also give rise to chronic diarrhea and neurological findings (2-4). High levels of ethylmalonic acid in the urine may also be observed inshort-chain acyl-CoA dehydrogenase deficiency and glutaric acidemia Type II, but none of these has been associated with the major clinical features of EE. Many patients have pyramidal or extrapyramidal signs, hypotonia, microcephaly, failure to thrive, seizures and episodes of coma. Combined treatment with oral metronidazole, N-acetylcysteine, riboflavinand coenzyme Q10 resulted in marked neurological improvement, disappearance of diarrhea and acrocyanosis in our patients (9,10). In conclusion, the possibility of EE should be investigated and examined in patients presenting with chronic diarrhea, early onset of neurological degeneration, recurrent petechiae and orthostatic acrocyanosis.

Ethics

Informed Consent: The parents of the children reported here have consented to these studies.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ç.S.K., S.C., Concept: Ç.S.K., Design: Ç.S.K., S.C., Data Collection or Processing: Ç.S.K., S.C., Analysis or Interpretation: Ç.S.K., S.C., Literature Search: Ç.S.K., Writing: Ç.S.K., A.A., E.P., M.K., S.C.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Bijarnia-Mahay S, Gupta D, Shigematsu Y, et al. Ethylmalonic Encephalopathy in an Indian Boy. Indian Pediatr 2016;53:914-6.
2. Yiş U, Polat İ, Karakaya P, Ayanoğlu M, Hiz AS. Importance of acrocyanosis indelayed walking. J Pediatr Neurosci 2015;10:80-1.
3. Henriques BJ, Lucas TG, Rodrigues JV, et al. Ethylmalonic encephalopathy ETHE1 R163W/R163Qmutations alterprotein stability and redox properties of the iron centre. PLoS One 2014;9:e107157.
4. Tiranti V, Zeviani M. Altered sulfide (H(2)S) metabolism in ethylmalonicencephalopathy. Cold Spring Harb Perspect Biol 2013;5:a011437.
5. Pavlou E, Augoustides-Savvopoulou P, Gregersen N, et al.An infant with ethylmalonic encephalopathymasquerading as a hematologic disorder. J Child Neurol 2013;28:668-71.
6. Dweikat I, Naser E, Damsah N, Libdeh BA, Bakri I. Ethylmalonic encephalopathyassociated with crescentic glomerulonephritis. Metab Brain Dis 2012;27:613-6.
7. Giordano C, Viscomi C, Orlandi M, et al. Morphologic evidence of diffusevascular damage in human and in the experimental model of ethylmalonicencephalopathy. J Inherit Metab Dis 2012;35:451-8.
8. Walsh DJ, Sills ES, Lambert DM, et al.Novel ETHE1mutation in a carrier couple having prior offspring affected with ethylmalonicencephalopathy: Genetic analysis, clinical management and reproductive outcome.Mol Med Rep 2010;3:223-6.
9. Palmfeldt J, Vang S, Stenbroen V, et al. Proteomics reveals that redoxregulation is disrupted in patients with ethylmalonic encephalopathy.J ProteomeRes 2011;10:2389-96.
10. Di Meo I, Fagiolari G, Prelle A, et al. Chronicexposure to sulfide causes accelerated degradation of cytochrome c oxidase inethylmalonic encephalopathy. Antioxid Redox Signal 2011;15:353-62.

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Case Report

A 6-Month-Old Boy with Reddish, Scaly Skin: Netherton Syndrome

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ABSTRACT

Typical features of Netherton syndrome are congenital ichthyosiform erythroderma, atopic diathesis and trichorrhexis nodosa. Here in this report, we present a case with congenital ichthyosis with atopy presenting later. We wanted to discuss the importance of whole exome sequencing to diagnose the atypical presentations of common syndromes.

Keywords: Ichthyosis, erythroderma, Netherton syndrome, atopy, whole exome sequencing

Introduction

Netherton syndrome (OMIM #256500) is an autosomal recessively inherited syndrome, first described by Netherton (1). Netherton syndrome is caused by homozygous or compound heterozygous mutations in the *SPINK5* gene, which encodes the serine protease inhibitor LEKTI (lympho-epithelial Kazal-type-related inhibitor), on chromosome 5q32 (2). Clinical features are congenital ichthyosiform erythroderma, atopic diathesis and specific bamboo hair appearance (3). Less than a hundred cases have been reported so far. However, atypical cases make the diagnosis difficult. We present a case of Netherton syndrome with congenital ichthyosis with atopy presenting later.

Case Report

A six-month-old boy was admitted to our hospital due to dry, reddish, scaly skin and failure to thrive. He was born by caesarean section at 38 weeks gestational age to a 26-year-old, G1P1, healthy woman. He weighed 2900 gr (appropriate for gestational age). He had respiratory distress soon after birth, was diagnosed with neonatal pneumonia and stayed at the neonatal intensive care unit for 15 days. His skin findings started when he was 1 week old and became worse in time, although the family applied some skin ointments and zinc suspensions. He was breastfed supplemented with infant formula. He had loose stools but did not have chronic diarrhea. The patient's parents were first degree cousins, other than this, his family history was unremarkable.

On physical examination, weight, height and head circumference were 3500 gr, 52 cm and 39 cm respectively (all below the third percentile for his age). His general appearance was well, he had normal motor and mental development. He had generalized, scaly erythroderma (Figure 1, informed consent was taken from patient's legal guardians). On auscultation, he had normal respiratory sounds, normal heart rate and no murmur. He had mild

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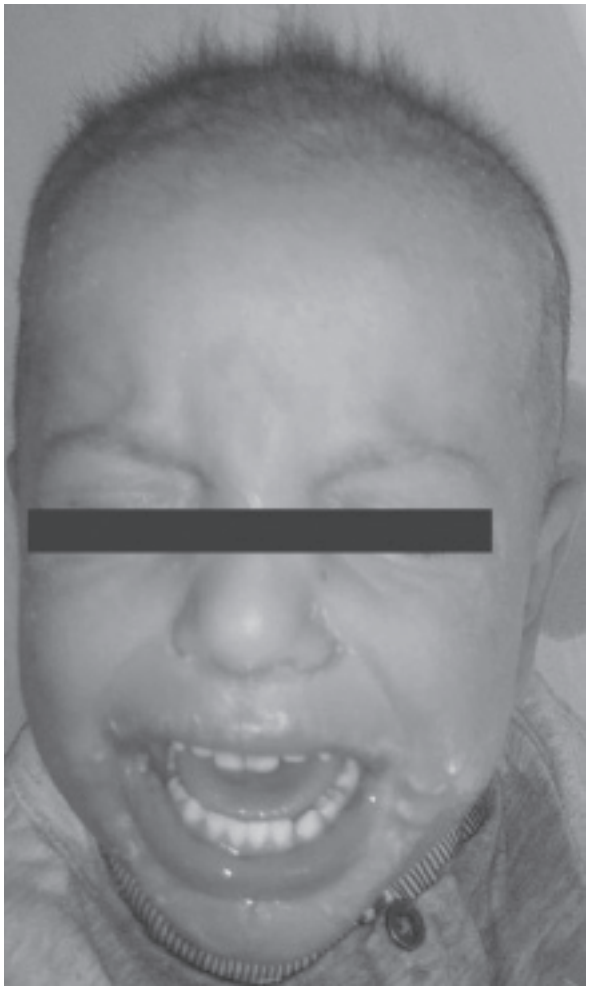

Figure 1. Scaly ichthyosiform erythroderma of patient's face, without apparent bamboo hair appearance

hepatosplenomegaly. He had no limb anomaly. He had sparse eyebrows and sparse scalp hair.

On laboratory investigation, the patient had mild anaemia (hemoglobin: 9.64 g/dL, hematocrit: 29.9%), normal liver transaminases (aspartate aminotransferase: 26 IU/L, alanine aminotransferase: 17 IU/L) and hypoalbuminemia (albumin: 2.3 g/dL). Thyroid function tests, anti-tissue transglutaminase and anti-endomysial antibodies were within normal limits for age. Serum immunoglobulin E (IgE) level was 841 IU/mL (normal: 10-180). Other Ig levels were within reference range for age (IgG: 1315 IU/mL, IgA: 127 IU/mL, IgM: 351 IU/mL). In order to investigate the underlying atopy, a skin prick test was done and revealed negative. The peripheral blood smear showed the prominence of eosinophilia (absolute eosinophil count: 12%). Differential diagnoses for this infant were autosomal recessive lamellar ichthyosis and harlequin ichthyosis. For autosomal recessive congenital ichthyosis and harlequin ichthyosis, molecular analyses of *TGM1*, *NIPAL4* and *ABCA12* genes were all normal. Since he had mild hepatosplenomegaly along with the skin findings, Gaucher disease Type II was suspected but molecular analysis of the *GBA* gene was normal. For a definite diagnosis, a whole exome sequencing was performed and detected a known, disease causing homozygous mutation in *SPINK5* gene [IVS2+5G>T (c.81+5G>T)]. The diagnosis was Netherton syndrome. Although the patient had a negative skin prick test, food specific IgE panel revealed that plasma levels of egg white specific IgE, milk specific IgE and wheat specific IgE were all high (65.5, 8.51 and 38.7 kUA/L respectively). Milk, wheat and egg white were all eliminated from his diet and his skin condition improved in the course of time. Food challenge tests for milk, wheat and egg white were all positive and these three foods were permanently eliminated from the diet. Concurrently, microscopically, his hair exhibited the typical bamboo hair appearance gradually and erythroderma resolved with the restricted diet. Netherton syndrome (OMIM #256500) is an autosomal recessive disorder, characterized by congenital ichthyosiform erythroderma, atopic diathesis and trichorrhexis nodosa (3). Rarely collodion babies are seen. The disorder is commonly confused with atopic dermatitis but does not respond to topical corticosteroid treatment (4). In the beginning, our patient was suspected of atopy but he had a negative skin prick test. Subsequently, food specific IgE panel and food challenge tests revealed the atopy. The false negative skin prick test was attributed to ichthyosis. Rarely, progressive and fatal hypernatremic dehydration may be seen in infants. In our patient, due to a coincidental neonatal pneumonia, our patient was well-hydrated in the neonatal intensive care unit and hypernatremic dehydration was not seen. His malnutrition, hypoalbuminemia and iron deficiency anaemia were attributed to enteropathy which is consistent with the syndrome (5). Diagnosis may be delayed beyond the neonatal period until the appearance of the pathognomonic bamboo hair anomaly which may also be recognized along the disease course (5). Under a light microscope, hair showing nodular trichorrhexis is diagnostic (the distal area of hair invaginates toward its proximal area). The histological findings of skin biopsy are frequently non-characteristic thinning of the granular layer and stratum corneum, psoriasiform hyperplasia and less common compact parakeratosis with large nuclei, subcorneum or intracorneum splitting, presence of clear cells in the upper epidermis or stratum corneum, dyskeratosis, dermal infiltrate with neutrophils and/or eosinophils and dilated blood vessels in the superficial dermis (6). On transmission electron microscopy, immature lamellar granules are observed between keratinocytes (7). Another non-invasive diagnostic method is molecular analysis of the *SPINK5* gene, this provides a genetic counselling opportunity to the family. Treatment is symptomatic and requires prompt management of the neonatal complications such as fluid and electrolyte management, an elimination diet to prevent atopic dermatitis and long-term use of emollients and/or topical immunomodulators for amelioration of the skin disorder (7,8). Some patients responded to ammonium lactate lotion, the per oral retinoid and psoralen plus ultraviolet A therapy. The prognosis may be severe in neonates with life-threatening complications like recurrent dermal infections, fluid and

electrolyte abnormalities and postnatal lethality is high. The skin manifestations and hair anomalies persist throughout life, but the disease usually improves with age and most patients begin to thrive during the second year of life (6,7).

Discussion

More than a hundred conditions are described relating to ichthyosis. It is important to make the specific diagnosis by detecting features other than skin findings in order to establish proper treatment, predict the prognosis and also provide genetic counselling for the family. Molecular studies can be helpful in making an accurate diagnosis and are also non-invasive.

Ethics

Informed Consent: Informed consent was taken from patient's legal guardians.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.D.B., N.Ö.M., Concept: F.D.B., N.Ö.M., Design: F.D.B., M.Y., D.U.A., N.Ö.M., Data Collection and Processing: F.D.B., Analysis and Interpretation: S.C., Literature Search: S.K., D.K., B.Ş.Y., Writing: F.D.B., S.K., D.K., B.Ş.Y., M.Y., D.U.A., S.C., N.Ö.M.

Conflict of Interest: There are no conflicts of interest.

Financial Disclosure: There is no financial support to disclose.

References

1. Netherton EW. A unique case of trichorrhexis nodosa: 'bamboo hairs'. Arch Derm 1958;78:483-7.
2. Chavanas S, Garner C, Bodemer C, et al. Localization of the Netherton syndrome gene to chromosome 5q32, by linkage analysis and homozygosity mapping. Am J Hum Genet 2000;66:914-21.
3. Bitoun E, Chavanas S, Irvine AD, et al. Netherton syndrome: disease expression and spectrum of SPINK5 mutations in 21 families. J Invest Derm 2002;118:352-61.
4. Smith DL, Smith JG, Wong SW, deShazo RD. Netherton's syndrome: a syndrome of elevated IgE and characteristic skin and hair findings. J Allergy Clin Immun 1995;95:116-23.
5. Fartasch M, Williams ML, Elias PM. Altered lamellar body secretion and stratum corneum membrane structure in Netherton syndrome: differentiation from other infantile erythrodermas and pathogenic implications. Arch Derm 1999;135:823-32.
6. Leclerc-Mercier S, Bodemer C, Furio L, et al. Skin Biopsy in Netherton Syndrome: A Histological Review of a Large Series and New Findings. Am J Dermatopathol 2016;38:83-91.
7. Tiryakioğlu NO, Önal Z, Saygili SK, Önal H, Ersoy Tunali N. Treatment of ichthyosis and hypernatremia in a patient with Netherton syndrome with a SPINK5 c.153delT mutation using kallikrein inhibiting ointment. Int J Dermatol 2017;56:106-8.
8. Yoneda K. Inherited ichthyosis: Syndromic forms. J Dermatol 2016;43:252-63.

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Case Report

Tyrosinemia Type I and Reversible Neurogenic Crisis After a One-Month Interruption of Nitisinone

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ABSTRACT

Hereditary tyrosinemia Type I (HTI) is an autosomal recessive disorder due to a deficiency of the enzyme fumarylacetoacetate hydrolase. The liver is the primary organ that is affected and comorbidities with renal and neurologic systems and hepatocellular carcinoma can be seen as a long-term complication. An effective treatment has been available with 2-[2-nitro-4-trifluoromethylbenzoyl]-1,3-cyclohexanedione (NTBC) since 1992. Neurogenic crises do not take place in HTI patients who are treated with NTBC. Here, we report on a seven-year-old boy who underwent a severe neurological crisis including anorexia, vomiting, weakness, hyponatremia, paresthesia and paralysis of the extremities, seizure and arterial hypertension after an interruption of NTBC treatment. With the re-introduction of NTBC, the patient gradually reacquired normal neurological functions, normal blood pressure and recovered completely.

Keywords: Tyrosinemia Type I, neurogenic crises, nitisinone

Introduction

Hereditary tyrosinemia Type I (HTI) (OMIM 276700) is a rare inborn error of the tyrosine metabolism due to a deficiency of the enzyme fumarylacetoacetate (FAA) hydrolase in the tyrosine catabolic pathway (Figure 1) (1). Biochemically, patients typically have hyper tyrosinemia and toxic metabolites. Toxic metabolites and their derivates such as FAA, maleylacetoacetate, succinyl acetoacetate and succinyl acetone (SA) play a major role in tissue damage with hepatic, renal and neurological findings. Before 2-[2-nitro-4-trifluoromethylbenzoyl]-1,3-cyclohexanedione (NTBC), over 90% of patients died before 12 years of age 10% of them were due to neurogenic crises with respiratory problems (2). A L-phenylalanine and tyrosine restricted diet was the only treatment. The introduction of NTBC about 25 years ago greatly enhanced survey and prognosis of HTI as it was effective within hours, eradicating hepatic and neurological findings and protecting from the risk of hepatocellular carcinoma when treatment starts within the first months of life (3). NTBC had been used as a herbicide. The mechanism of NTBC is as an inhibitor of 4-hydroxyphenylpyruvate dioxygenaseis to block tyrosine catabolism at an initial step and convert HTI into Type III tyrosinemia. This hinders the production of toxic metabolites which are responsible for the hepatic, renal and neurological involvements of these toxic products, SA was discovered to curtail the activity of the enzyme delta 5-aminolevulinic acid dehydratase in the heme pathway (Figure 1). Thus, neurogenic crises in HTI have a physiological base fundamentally similar to those occurring in porphyria and lead poisoning, in which delta 5-aminolevulinic acid is also heightened. The clinical courses of these neurogenic crises also resemble Guillain-Barré syndrome. Porphrya-like syndrome is usually precipitated by an intercurrent infection or interruption of NTBC. These

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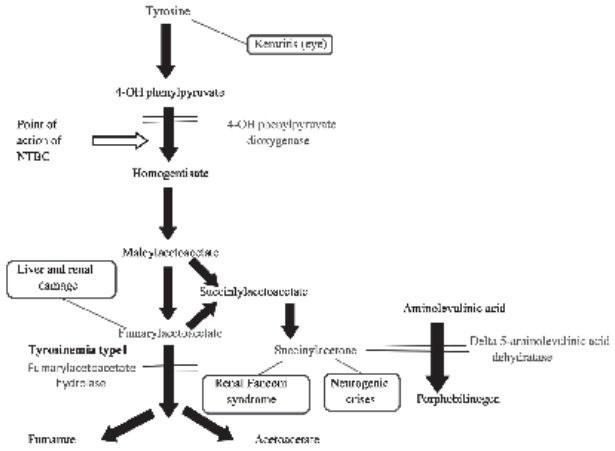

Figure 1. Patway of tyrosine metabolism in tyrosinemia Type I

crises with severe progression are characterized initially by pain (including abdominal pain resembling an acute surgical emergency), weakness and autonomic changes such as hypertension and hyponatremia. Patients may display an acute progressive ascending motor neuropathy, with or without hypertonic posturing, self-mutilation and convulsion. If this rarely seen complication is not diagnosed and treated early, it can be fatal. In a longitudinal study of HTI patients, no patient developed a neurogenic crises while being treated with NTBC (4). We report on a seven year-old boy with a severe neurological crisis including anorexia, vomiting, weakness, hyponatremia, paresthesia and paralysis of the extremities, seizure and arterial hypertension after a one-month interruption of NTBC treatment. The patient slowly regained normal neurological functions and normal blood pressure and recovered completely with the re-introduction of NTBC.

Case Report

A boy at the age of seven was referred to the emergency room with abdominal pain, vomiting and weakness. The child was born at term as a first child of non-consan¬guineous parents with a normal birth weight and length. When he was eight months old, he had hepatosplenomegaly, rickets and hypotonia. He was diagnosed with HTI due to elevated plasma tyrosine and urine SA levels. The patient was immediately put on a restricted phenylalanine and tyrosine diet in conjunction with NTBC. Under this treatment by diet and NTBC (1-2 mg/kg/d), the boy developed normally until the age of seven years without any signs of growth, hepatic, renal or neurological deteriorations and never necessitated hospitalization. On physical examination in the emergency service room, he presented weight: 36 kg (90th percentile), height: 134 cm (90th percentile), blood pressure: 148/114 mm hg, compatible with phase 2 hyper tension, fever 37 °C, respiration: 30/min and pulse: 120/min. He looked to be anxious and ill and was in a lateral knee-chest position due to tenderness in the abdomen; nevertheless, defence, rebound, organomegaly and ascites were not observed. Deep tendon reflex examination was normal and there was no parasthesis or pathologic reflex. Laboratory analysis revealed normal glucose, hepatic and renal function tests and acute phase reactants. Abdomen ultrasonography and plain X-ray were performed and no significant findings were determined except for increased bowel gas pattern. Since hyponatremia (Na: 121 mEq/L, N: 136-145) was determined, intravenous saline solution was given. Intravenous glucose was also initiated as an energy source and to block the step before the hydratase (delta-aminolevulinic synthase). Amlodipine treatment was initiated since hypertension was detected with recurrent measurements. At the seventh hour of emergency service follow-up, generalized tonic-clonic seizures were observed followed by hypertonic posture. Antiepileptic treatment was initiated with intravenous dormicum and levetiracetam. We obtained a detailed medical history and learned the truth about the 30-day interruption of NTBC. The patient was evaluated as being in neurogenic crisis and admitted to the pediatric intensive care unit (PICU). At the time of admission to the PICU, furosemide and atenolol treatments were added as the hypertension and Glasgow Coma scale (GCS) of the patient was E3M4V1. At the 24th hour of hospitalization NTBC was reinitiated at a dosage of 2 mg/kg/d. After NTBC was re-administered, neurogenic crisis including seizures, progressive ascending polyneuropathy, hypertonic posture and respiratory distress requiring bilevel positive airway pressure support settled down. Consequently, we did not use haem arginate. Following PICU support of 36 hours, the patient was transferred to the inpatient clinic. At the time of transfer to the inpatient clinic, GCS was E4M6V5 and no seizure was observed during the inpatient clinic follow-up. Antihypertensive treatment was reduced gradually. The patient was discharged from the hospital without any symptoms after six days inpatient clinic follow-up. Amlodipine treatment was gradually reduced and ceased within three weeks, however levetiracetam was used for five months and then ceased. At present, the patient continues the diet and NTBC therapy.

Informed consent was obtained from the patient's parents.

Discussion

NTBC was utilized for the treatment of HTI in conjunction with a tyrosine restricted diet. It is an inhibitor of 4-hydroxyphenylpyruvate dioxygenase, and thus, this prevents the formation of toxic metabolites such as SA which have been shown to block the of delta 5-aminolevulinic acid dehydratase in the heme biosynthesis. Neurogenic crises in HTI have a physiological basis essentially similar to those occurring in porphyria and lead poisoning in which delta 5-aminolevulinic acid is also increased. The clinical course of neurogenic crises also resembles Guillain-Barré syndrome. Therefore, when HTI patients are admitted with nonspecific symptoms like irritability, pain, weakness, hypertension and

hyponatremia such as our patient, neurogenic crisis should be evaluated as well in order to improve the chance of a correct diagnosis. Before the NTBC era began about 25 years ago, with dietary treatment alone, over 90% of patients died before 12 years of age and 10% of these deaths were caused by neurogenic crises (2). Prior to NTBC, neurogenic crises could emerge at any time and age, particularly crises followed a minor infection. During the NTBC era, severe neurogenic crises may appear when NTBC treatment is interrupted (4-6). In a review of the literature, it can be seen that there are few reports on neurogenic crises in HTI patients following NTBC coming into use. Schlump et al. (5) reported an 8-month-old male who had a severe neurogenic crisis with progressive ascendant polyneuropathy, diaphragm paralysis and arterial hypertension after an interruption of NTBC for 2 months. All neurological signs and symptoms in question disappeared after a resumption of NTBC treatment (5). Neurogenic crises are only currently a problem in some countries owing to a lack of family awareness and health service problems. In 2016, Onenli Mungan et al. (7) reported a nine-month-old boy who had an irreversible neurological crisis after a one-month discontinuation of NTBC and they hypothesized that the duration of NTBC discontinuation is not the only factor determining the reversibility of neurogenic crisis. This again emphasizes the importance of continued patient compliance and that neurogenic crises are only a current problem because of a lack of family adherence to the treatment and health service problems. Our report showed that for HTI patients with nonspecific findings such as vomiting, weakness, hyponatremia and paresthesia or paralysis of the extremities, seizure and arterial hypertension, neurogenic crises should be considered at the outset.

Ethics

Informed Consent: Informed consent was obtained from the patient's parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.Y., E.E., M.A.K., Concept: H.Y., S.K.U., Design: H.Y., E.C., B.K., M.Ç., Data Collection and Processing: H.Y., Analysis and Interpretation: H.Y., Literature Search: E.E., Writing: H.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declare that this study received no financial support.

References

1. Chakrapani A, Gissen P, McKierrnan P. Disorders of tyrosine metabolism. In: Saudubray JM, van den Berghe G, Walter JH (eds). Inborn metabolic diseases, diagnosis and treatment, 6th ed. Heidelgerg, Springer 2016:265-76.
2. van Spronsen FJ, Thomasse Y, Smit GP, et al. Hereditary tyrosinemia Type I: A new clinical classification with difference in prognosis on dietary treatment. Hepatology 1994;20:1187-91.
3. Chinsky JM, Singh R, Ficicioglu C, et al. Diagnosis and treatment of tyrosinemia type I: a US and Canadian consensus group review and recommendations. Genet Med 2017:19.
4. Larochelle J, Alvarez F, Bussières JF, et al. Effect of nitisinone (NTBC) treatment on the clinical course of hepatorenal tyrosinemia in Québec. Mol Genet Metab 2012;107:49-54.
5. Schlump JU, Perot C, Ketteler K, et al. Severe neurological crisis in a patient with hereditary tyrosinaemia type I after interruption of NTBC treatment. J Inherit Metab Dis 2008;31(Suppl 2):223-5.
6. Zeybek AC, Kiykim E, Soyucen E, et al. Hereditary tyrosinemia type 1 in Turkey: twenty year single center experience. Pediatr Int 2015;57:281-9.
7. Onenli Mungan N, Yıldızdaş D, Kör D, et al. Tyrosinemia type 1 and irreversible neurologic crisis after one month discontinuation of nitisone. Metab Brain Dis 2016;31:1181-3.

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Case Report

Glutaric Aciduria Type I Diagnosis Case with Normal Glutaryl Carnitine and Urine Organic Acid Analysis

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ABSTRACT

Glutaric aciduria Type I (GA-I) is a rare inherited metabolic disease, deficiency of glutaryl-CoA dehydrogenase results in accumulation of the putatively neurotoxic metabolites glutaric and 3-hydroxyglutaric acid (GA, 3-OH-GA) in body tissues, particularly within the brain. Here we presented a 3-year-old girl with hypotonia and dystonia diagnosed with GA-I although the repeated analysis of the carnitine profile and organic acid analyses were normal. The patient has motor, mental retardation, hypotonia. Her weight standard deviation score (SDS) was -1.86 SDS, height SDS was -0.55 SDS, head circumference SDS was -1.01. The physical examination was normal except severe hypotonia. Spot blood carnitine profile, blood amino acid, urine organic acid, lactic acid and pyruvic acid were normal in repeated analysis. Dystonia and spastic tetraparesis developed on her follow-up. Cranial magnetic resonance imaging revealed bilateral cortical atrophy and bilateral striatal and caudate nucleus T2 flair hyperintensities. In *GCDH* gene analysis p.Y123C (c.368A>G)/p.L340F (c.368A>G) mutation was found. There was no history of encephalopathy. The patient treated with levodopa and trihexyphenidyl and lysine-restricted diet. In the presence of bilateral striatal involvement and cortical atrophy and dystonia, GA-I should be kept in mind. Blood carnitine profile and urine organic acid analyses may not be consistent. It is important to evaluate the cases for genetic investigation.

Keywords: Glutaric acid, glutaryl carnitine, dystonia

Introduction

Glutaric aciduria Type I (GA-I, OMIM 231669) is a rare metabolic disorder of autosomal recessive inheritance caused by deficient or nonfunctional glutaryl-coenzyme A (CoA) dehydrogenase (GCDH). The *GCDH* gene is localized on chromosome 19p13.2 and encodes the protein that is involved in the catabolism of L-lysine, L-hydroxylysine and L-tyrptophan (1,2). Acute neurological deterioration occurs most frequently between 6 and 18 months usually triggered by febrile illnesses or immunization. Movement disorder, dystonia, seizures and extrapyramidal symptoms are presented (3,4). In few patients, neurological disease has been demonstrated in the absence of any encephalopathic crisis termed as insidious-onset type (5) and late onset type (6). Biochemically GCDH deficiency is characterized by a deposition of glutaric acid (GA, 3 hydroxyglutaric acid (3-OH-GA), glutaconic acid (less frequently) and glutaryl carnitine (C5DC) (7). The major therapeutic principles GA-I are; the reduction of glutaryl CoA, GA, 3-OH-GA using low lysine diet and prevention of secondary carnitine deficiency (5). Here we presented a 4-year-old girl with hypotonia and dystonia and diagnosed with GA-I although the repeated analysis of the carnitine profile and organic acid analyses were normal.

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Case Report

Four years old girl was admitted to our clinic due to motor, mental retardation and hypotonia at the age of one year. She was born via vaginal delivery at 39th gestational week, with 2900 g birth weight as the first child of nonconsanguineios parents. On her first pediatric neurology visit at the age of 11 months' global developmental delay was detected. Her weight was 7.2 kg [-1.86 standard deviation score (SDS)] and height was 72 cm (-0.55 SDS) and head circumstance was 44 cm (-1.01 SDS). She was treated with physical therapy. When she was 18 months old, dystonia was detected. On physical examination severe hypotonia, and dystonia were detected, deep brain tendon reflexes were increased, and other systems were normal. Her eye examination was normal, and there was no hearing loss. There was no history of encephalopathy. Complete blood count results were in normal range. Renal function tests, liver function tests and creatine kinase levels were also normal. Spot blood carnitine profile, blood amino acid, urine organic acid, lactic acid and pyruvic acid were normal in repeated analyses. Cranial magnetic resonance imaging (MRI) revealed bilateral cortical atrophy and bilateral striatal and caudate nucleus T2 flair hyperintensities (Figure 1). Electromyogram analysis was normal. Feeding difficulties, hyper salivation and spastic tetra paresis developed on her follow-up. Cerebrospinal fluid (CSF) analysis performed and CSF glycine 79.1 µmol/L, blood glycine 379.9 µmol/L and CSF glycine/blood glycine ratio was high (0.2). CSF serine, blood serine, CSF lactate and blood lactate were all normal. Due to the suspicion of non ketotic hyperglycinemia (NKH), Illumina Inherited Disease Sequencing Panel analysis performed and it was negative for *NKH* genes (glycine decarboxylase-GLDC, aminomethyltransferase, glycine cleavage system H protein-*GCSH* genes). In *GCDH* gene analysis p.Y123C (c.368A>G)/p.L340F (c.368A>G) mutation was found (Figure 2). Her mother has heterozygous p.L340F (c.368A>G) mutation and her father has heterozygous p.Y123C (c.368A>G) mutation. After she had the diagnoses with GA-I, we repeated the CSF analysis and determined the normal levels of CSF and also blood glycine levels. The patient was treated with levodopa and trihexyphenidyl and lysine-restricted diet. Baclofen and diazepam treatment also given during follow up. Her last visit was at the age of 45 months. Failure to thrive was detected. Her weight was 9.6 kg (-2.78 SDS), her height was 89 cm (-4.6 SDS) and head circumstance 47 cm (-2.32 SDS). Spastic tetra paresis and dystonia was detected. During follow up all urine organic acid analyses were normal. Only in one sample spot blood glutaryl carnitine level was 0.49 µmol/L (n=0-0.35) slightly elevated. We also detected high levels of creatine kinase levels (1422 IU/L; n=34-145) which was returned to normal levels during follow up. During follow up clinical progression was observed and control cranial MRI investigation was performed. Findings were similar when compared with previous cranial MR.

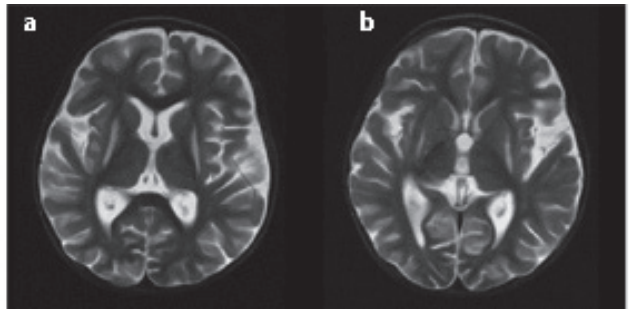

Figure 1. a, b) Axial T2, flair images show bilateral temporal atrophy and bilateral striatal hyperintensities

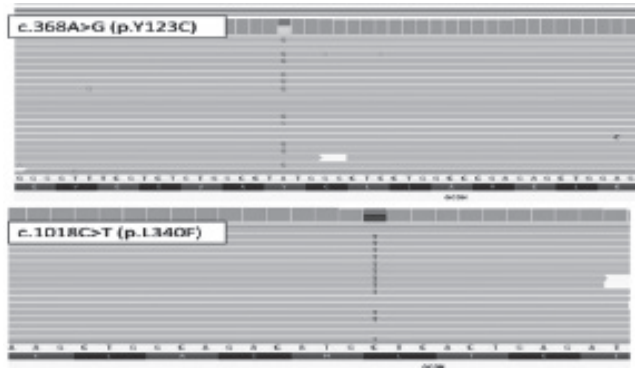

Figure 2. Molecular analysis of the patient

Discussion

GA-I is a rare neurometabolic disease and estimated prevalence is 1:110000 newborns (8). For most patients with GA-I, the acute encephalopathic crisis occurs and usually triggered by an infection or vaccination (3,4). Recent studies suggest that neurological damage may occur in the absence of encephalopathic crisis and has been termed insidious-onset (9). In our patient there was no history of acute encephalopathic crisis, but progressive neurodegeneration was detected. Macrocephaly is found 75% of the patients with GA-I, but is non-specific (9) Also macrocephaly was absent in our patient. In the literature it was shown that epilepsy is frequent in patients with GA-I (8) unlikely our patient had no seizure. Like to the literature dystonia was the dominant extrapyramidal symptom in our patient (9). CSF analysis was performed in our patient due to presence of dystonia. High CSF glycine level and high CSF glycine/plasma glycine ratio was detected. Movement disorders may present in late onset NKH patients (10). But in our patient's dystonia was started at 18 months at an early age and control CSF glycine levels detected within normal range. We could not find any explanation for this issue. She had no seizure and no treatment when CSF sample was taken. So we ruled out NKH by genetic analysis. Urine organic acid analysis usually shows increased levels of GA, 3-OH-GA, glutaryl-CoA, glutarylcarnitine although GA and dicarboxylic carnitines can be completely normal in some patients (11). In GA-I; brain GA has been found to exceed plasma and CSF

levels even in patients with normal or low GA levels in urine, best explained by Kölker et al (3). It has been shown that urine C5DC, GA and 3-OH-GA did not show any correlation with the patients' clinics (3,12). We had not performed urine C5DC, but all urine organic acid analyses were normal during follow up. Only one spot blood carnitine analysis, slightly high glutaryl carnitine level was detected. We want to mention that normal blood carnitine and urine organic acid analysis can be found in patients with GA-I. Cranial MRI plays an important role in the diagnostic work-up in GA-I patients. Many characteristic findings have been reported focusing on striatal changes, subdural collection and widening Sylvian fissures, abnormal signal intensity of caudate and putamen. Abnormalities of other gray matter structures have been rarely reported (12). The MRI findings of our patient such as striatal hyperintensities and temporal atrophy are common in GA-I patients. Our patient had the diagnosed by Illumina Inherited Disease Sequencing Panel analysis. *GCDH* gene analysis revealed p.Y123C (c.368A>G) mutation on one allele and p.L340F (c.1018C>T) mutation on the other allele. To the best of our knowledge this mutation has not been published in the literature.

Basic metabolic treatment of GA-I is using low lysine diet for reduction of glutaryl-CoA, GA and 3-OH-GA and carnitine supplementation to prevent carnitine depletion. Riboflavin treatment is less frequent (5). Carnitine status and plasma amino acid concentrations should be monitored in GA-I patients (9). The value of metabolic treatment is unclear in patients with severe neurological deterioration, however it may be beneficial to the progression of the symptoms (9). Dietary treatment in combination with carnitine and emergency treatment has been shown to be effective in preventing neurological disease (5). We treated our patient with low lysine diet and carnitine supplementation. During follow up, spot blood free carnitine levels of the patient were normal. Also, repeated urine organic acid analyses were all normal. With the basic metabolic treatment, we didn't detect progression of the patients' symptoms but also no beneficial effect. GA-I is a treatable inborn metabolic disorder. It is important including GA-I in the differential diagnosis of leukoencephalopathy, dystonia combined with macrocephaly or not. The encephalopathic crisis may be absent and glutaryl carnitine elevation and urine glutaric acid elevation can not be detected. The diagnosis should be suspected on the combination of clinical and neuroradiological findings of the patient and GCDH gene analysis should be performed in such cases.

Ethics

Informed Consent: Informed concent form was filed out by all participants.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.C., M.Ç., Concept: E.C., Y.A.A., Design: M.Ç., S.K.U., Data Collection or Processing: H.M.S., G.S., C.E., Analysis or Interpretation: H.O., F.Ö., S.H., Literature Search: H.Y., E.E. Writing: E.C., S.K.U.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Goodman SI, Markey SP, Moe PG, Miles BS, Teng CC. Glutaric aciduria; a "new" disorder of amino acid metabolism. Biochem Med 1975;12:12-21.
2. Koeller DM, DiGiulio KA, Angeloni SV, et al. Cloning, structure, and chromosome localization of the mouse glutaryl-CoA dehydrogenase gene. Genomics 1995;28:508-12.
3. Kölker S, Garbade SF, Greenberg CR, et al. Natural history, outcome, and treatment efficacy in children and adults with glutaryl-CoA dehydrogenase deficiency. Pediatr Res 2006;59:840-7.
4. Bjugstad KB, Goodman SI, Freed CR. Age at symptom onset predicts severity of motor impairment and clinical outcome of glutaric acidemia type 1. J Pediatr 2000;137:681-6.
5. Kölker S, Christensen E, Leonard JV, et al. Guideline for the diagnosis and management of glutaryl-CoA dehydrogenase deficiency (glutaric aciduria type I). J Inherit Metab Dis 2007;30:5-22.
6. Külkens S, Harting I, Sauer S, et al. Late-onset neurologic disease in glutaryl-CoA dehydrogenase deficiency. Neurology 2005;64:2142-4.
7. Baric I, Wagner L, Feyh P, Liesert M, Buckel W, Hoffmann GF. Sensitivity and specificity of free and total glutaric acid and 3-hydroxyglutaric acid measurements by stable-isotope dilution assays for the diagnosis of glutaric aciduria type I. J Inherit Metab Dis 1999;22:867-81.
8. Boy N, Mühlhausen C, Maier EM, et al. Proposed recommendations for diagnosing and managing individuals with glutaric aciduria type I: second revision. J Inherit Metab Dis 2017;40:75-101.
9. Kölker S, Christensen E, Leonard JV, et al. Diagnosis and management of glutaric aciduria type I--revised recommendations. J Inherit Metab Dis 2011;34:677-94.
10. Lee WT. Disorders of amino acid metabolism associated with epilepsy. Brain Dev 2011;33:745-52.
11. Pineda M, Ribes A, Busquets C, Vilaseca MA, Aracil A, Christensen E. Glutaric aciduria type I with high residual glutaryl-CoA dehydrogenase activity. Dev Med Child Neurol 1998;40:840-2.
12. Couce ML, López-Suárez O, Bóveda MD, et al. Glutaric aciduria type I: outcome of patients with early- versus late-diagnosis. Eur J Paediatr Neurol 2013;17:383-9.

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Letter to the Editor

Successful Management of Ornithine Transcarbamylase Deficiency Presenting with Reversible Metabolic Stroke in a Child

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Keywords: Metabolic stroke, ornithine transcarbamylase deficiency, successful treatment

Dear Editor;

Ornithine transcarbamylase deficiency (OTC) is the most common urea cycle disorder with an estimated prevalence of 1 per 80000 births. In a genetic alteration of ornithine transcarbamylase, enzyme in the hepatic mitochondria leads to the accumulation of ammonia and its metabolites (1). It usually causes hyperammonemic encephalopathy in males during the neonatal to infantile period whereas female carriers present with variable manifestations depending on their pattern of random chromosome X inactivation or spontaneous mutations (2). Hyperammonemia represents a medical emergency requiring prompt treatment to prevent severe neurological damage, coma, and death. We present a rare cause of metabolic stroke due to late onset OTC deficiency that has been successfully managed with a combined therapy of sodium benzoate, arginine and a protein-restricted diet. No neurological damage was apparent at 9 months after treatment. A 6-year-old girl presented with vomiting for 6 months that was accompanied by episodes of anxiety and intermittent irritability. She was born at term with a birth weight of 3.9 kg to nonconsanguineous healthy parents. Antenatal history and neonatal period were unremarkable. Mental and motor development was normal. On neurological examination, she was anxious, disorientated, markedly agitated, and unable to follow commands. There was no fever, meningism or focal neurological signs. The remainder of the examination was normal. We learned that protein intake was inadequate for her age, that she had been fed only milk for the last week and also 5 months before she had been taken to hospital with the same complaints, getting the diagnosis of probable viral encephalitis, cerebrospinal fluid analysis, neuroimaging and electroencephalogram had been reported as normal. Laboratory investigations including serum glucose, electrolytes, thyroid stimulating hormone, free thyroxine, lactate, pyruvate, erythrocyte sedimentation rate, C-reactive protein, renal and liver function tests were all

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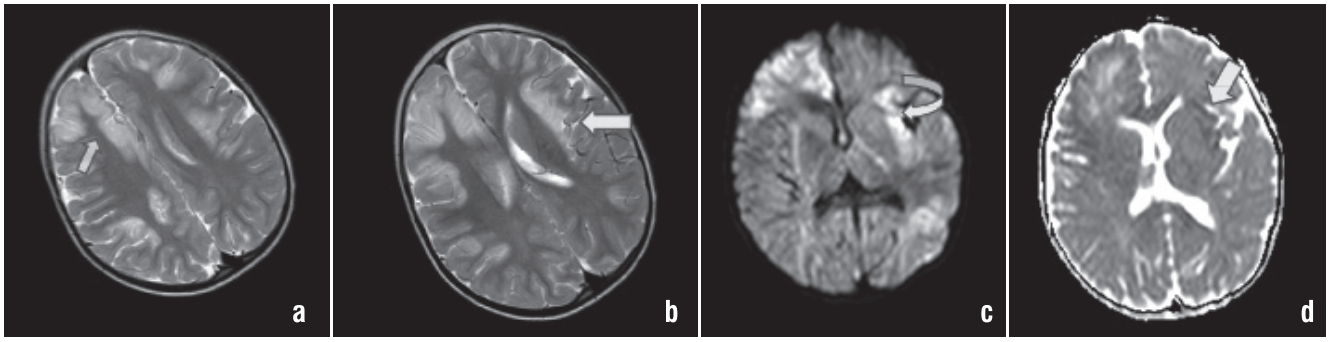

Figure 1. a, b) Axial FLAIR images [TR (repetition time)/TE (echo time); 3000/100 ms)] show bilateral lesions of insulae, frontal lobes and cingulate gyrus. c, d) images and axial isotropic trace diffusion images (TR/TE; 6600/89 ms with a maximum b value of 1000 s/mm^2) reveal restricted diffusion pattern corresponding apparent diffusion coefficient maps reveal low values in the lesions

within reference ranges. The initial ammonia level illustrated 368 µmol/L (reference interval: 16-47 µmol/L). Brain magnetic resonance (MR) imaging revealed bilateral lesions of the insulae, frontal lobes and cingulate gyrus on axial T2-weighted images and corresponding apparent diffusion coefficient maps revealed low values in the lesions considering a metabolic stroke (Figure 1). Plasma homocycteine level was 8 µmol/L (reference interval: 5.0 and 15.0 µmol/L) and the result of cardiac examination was normal. After the exclusion of acquired disorders as the explanation for the hyperammonemia (infections and toxic reasons), the suspicion of a urea cycle defect became stronger in the light of the anamnesis. Metabolic studies obtained before the initiation of therapy were notable for significantly elevated urine orotic acid in organic acid analysis. Blood amino acid analysis conducted simultaneously revealed an increase in glutamine and a decrease in arginine, citrulline. Intravenous dextrose emulsion together with sodium benzoate (250 mg per kg bodyweight per day), arginine and a protein-restricted diet were started considering OTC deficiency. Within 72 h she was conscious and feeling better with no permanent sequelae. She was discharged on oral sodium benzoate, phenylbutarate, L-citrulline and arginine. A known nonsense spontaneous mutation c.67C>T (p.Arg23*) was detected in exon 1 at heterozygous state at *OTC* gene. Her sister and parents were not carriers for the above mentioned mutation found in our patient. Glutamine peak on brain MR spectroscopy supported our diagnosis and the follow-up MR imaging performed 3 months later indicated hyperintense areas in both insulae and frontal lobes on axial T2-weighted images (Figure 2). Her metabolic situation remained stable, and she had no attacks for 9 months, keeping a normal physical and neurological state. Metabolic disorders (urea cycle disorders, branched-chain organic acidurias, mitochondrial diseases) are rare causes of stroke in children (3), which can present with various symptoms including vomiting, lethargy, seizures, coma. The heterogeneity of transience and onset in late-onset OTC deficiency often delay the diagnosis. Several clues such as voluntary protein avoidance and episodic feature of attacks can help to diagnose the condition during the asymptomatic period. Especially in a catabolic state

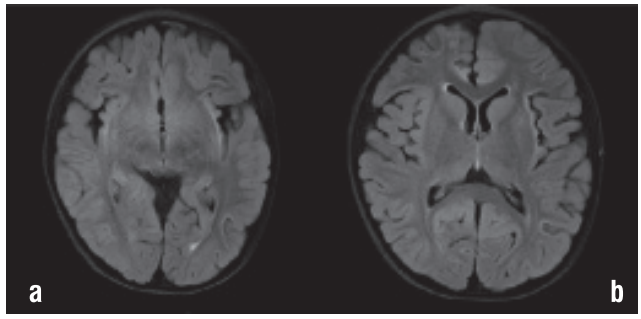

Figure 2. a, b) Axial FLAIR images (repetition time/echo time; 3000/100 ms) performed 3 months later indicated hyperintense areas in both insulae and frontal lobes

ammonia levels can rise quickly causing behavioural changes and vomiting, similar to our case (2). Upon a more thorough anamnestic evaluation, our patient's mother reported voluntary protein avoidance of the child. Excessive protein load is a trigger of an attack which is a specific trigger for urea cycle defects. Hyperammonemia and glutamate mediated excitotoxicity have been proposed as the main factor in the pathophysiology of impaired consciousness and seizures but stroke-like episodes in OTC deficiency may or may not be correlated with serum ammonia levels. The formation of glutamine from ammonia and glutamate in cerebral astrocytes leads to increased intracellular osmolarity, cell loss and cytokine release. Cytotoxic cerebral oedema causes impaired cerebral autoregulation and an increase in cerebral blood flow that results in diffusion restricted stroke-like white matter, insular and perirolandic region lesions (4,5). A study which investigated the difference of infarction distribution between the metabolic stroke and non-metabolic stroke group reported that metabolic stroke had no particular influence on anterior circulation or posterior circulation and the most common infarction pattern was deep infarction and border zone infarction (6). Urea cycle defects should be considered in the presence of history and neuroimaging findings. Our patient's brain MR imaging indicated T2 hyperintense lesions of insulae, frontal lobes, cingulate gyrus with restricted diffusion pattern that supported metabolic stroke. Similar to our case a female patient with OTC deficiency had been

treated with a combined therapy of continuous venovenous hemodialysis and N-carbamylglutamate. Her computed tomography examination performed a few weeks after the coma revealed a moderate hypoperfusion of the cerebral cortex without significant perfusive focal defects (7). Mutation analysis revealed a known nonsense spontaneous mutation c.67C>T (p.Arg23*) in exon 1 at heterozygous state at *OTC* gene in our patient (8). In the literature the nonsense mutation was detected in a 13-year-old Japanese girl who presented with an episode of irritability and vomiting (9). The majority of identified mutations reported for OTC deficiency are amino acid replacements. G-to-A and C-to-T transitions are the most frequent substitutions as in our case. Nonsense spontaneous mutations could be responsible in late onset OTC deficiency whereas one-third of the mutations in X-linked lethal disorders most probably postulated due to *de novo* events. It is suggested that OTC deficiency is a highly pleomorphic disorder in which other genetic and environmental modifiers play an important role (10-12). Mortality and morbidity rates are high in hyperammonemic encephalopathy in children with OTC deficiency, so aggressive management should be prompt as the prognosis is strongly influenced by the duration of the coma and peak ammonia levels. Long-term management with arginine and L-citrulline supplementation is debated to help to decrease the frequency of attacks and improve physical growth (13,14). Consequently late-onset OTC deficiency should be considered in the differential diagnosis of stroke, which is a rare presentation of a treatable disorder and early diagnosis can prevent a fatal outcome. Dietary habits and the recurrence of attacks could be helpful for the early diagnosis of urea cycle disorders with unusual presentations.

Ethics

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: Ö.D., Design: Ö.D., A.A., Data Collection or Processing: Ç.S.K., Ö.D., Analysis or Interpretation: A.A., K.K.O., Literature Search: K.K.O., Ç.S.K., E.A., Writing: Ö.D., A.A.

Conflict of Interests: The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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References

1. Nakamura K, Kido J, Matsumoto S and et al. Clinical manifestations and growth of patients with urea cycle disorders in Japan. J Hum Genet 2016;61:613-6.
2. Choi JH, Lee BH, Kim JH and et al. Clinical outcomes and the mutation spectrum of the OTC gene in patients with ornithine transcarbamylase deficiency. J Hum Genet 2015;60:501-7.
3. Kalidas K, Behrouz R. Inherited metabolic disorders and cerebral infarction. Expert Rev Neurother 2008;8:1731-41.
4. Braissant O, Mc Lin VA, Cudalbu C. Ammonia toxicity to the brain. J Inherit Metab Dis 2013;36:595-612.
5. Gropman A. Brain imaging in urea cycle disorders. Mol Genet Metab 2010;1:20-30.
6. Zhang Z, Xiao M, Ye Zandetal. Noncardiogenic stroke patients with metabolic syndrome have more border-zone infarction and intracranial artery stenosis. J Stroke Cerebrovasc Dis 2015;24:629-34.
7. Tummolo A, Favia V, Bellantuono R and et al. Successful early management of a female patient with a metabolic stroke due to ornithine transcarbamylase deficiency. Pediatr Emerg Care 2013;29:656-8.
8. Grompe M, Caskey CT, Fenwick RG. Improved molecular diagnostics for ornithine transcarbamylase deficiency.Am J Hum Genet 1991;48:212-22.
9. Matsuda I, Tanase S. The ornithine transcarbamylase (OTC) gene: mutations in 50 Japanese families with OTC deficiency.Am J Med Genet 1997;71:378-83.
10. Hata A, Setoyama C, Shimada K and et al. Ornithine transcarbamylase deficiency resulting from a C-to-T substitution in exon 5 of the ornithine transcarbamylase gene. Am J Hum Genet 1989;45:123-7.
11. Caldovic L, Abdikarim I, Narain S and et al. Genotype–Phenotype Correlations in Ornithine Transcarbamylase Deficiency: A Mutation Update. J Genet Genomics 2015;42:181-94.
12. McCullough BA, Yudkoff M, Batshaw ML and et al.Genotype spectrum of ornithine transcarbamylase deficiency: correlation with the clinical and biochemical phenotype. Am J Med Genet 2000;93:313-9.
13. Oscarson M, Vassiliou D, Nordenström A. Hereditary metabolic diseases with onset in adulthood. Early and correct treatment of acute symptoms can be life-saving. Lakartidningen 2016;1:113.
14. Bergmann KR, McCabe J, Smith TR and et al. Late onset ornithine transcarbamylase deficiency: treatment and outcome of hyperammonemic crisis. Pediatrics 2014;133:1072-6.

Ege University Children's Hospital Metabolic (Enzymology) Laboratory Analysis List- 1		
Enzyme Analysis	**Fabry disease:** alpha galactosidase	Dried blood sample: 2 mL (1tube containing EDTA) Leukocyte analysis: 4 mL (2 tubes containing EDTA) Plasma: 2 ml blood (1 tube containing EDTA)
	Gaucher Disease: Beta glucosidase	Dried blood sample: 2 mL (1tubecontaining EDTA) Leukocyte analysis: 4 mL (2 tubes containing EDTA)
	Niemann Pick A-B Disease: sphingomyelinase	Dried blood sample: 2 mL (1tubecontaining EDTA)
	GM1 gangliosidosis/MPS Type IV B: beta galactosidase	Dried blood sample: 2 mL (1tubecontaining EDTA) Leukocyte analysis: 4 mL (2 tubes containing EDTA)
	Tay Sachs–GM2 Disease: Beta hexosaminidase A	Dried blood sample: 2 mL (1tubecontaining EDTA) Leukocyte analysis: 4 mL (2 tubes containing EDTA)
	Sandhoff–GM2 Disease: Beta hexosaminidase A+B	Dried blood sample: 2 mL (1tubecontaining EDTA) Leukocyte analysis: 4 mL (2 tubes containing EDTA)
	Krabbe Disease: Beta galactocerebrosidase	Dried blood sample: 2 mL (1tubecontaining EDTA)
	MPS Type I: Alpha-L- iduronidase	Dried blood sample: 2 mL (1tubecontaining EDTA) Leukocyte analysis: 4 mL (2 tubes containing EDTA)
	MPS Type II: iduronidate 2 sulphatase	Dried blood sample: 2 mL (1tubecontaining EDTA) Leukocyte analysis: 4 mL (2 tubes containing EDTA)
	MPS Type VII: Beta glucuronidase	Dried blood sample: 2 mL (1tubecontaining EDTA) Leukocyte analysis: 4 mL (2 tubes containing EDTA)
	Alpha Mannosidosis: Alpha mannosidase	Dried blood sample: 2 mL (1tubecontaining EDTA) Leukocyte analysis: 4 mL (2 tubes containing EDTA)
	Fucosidosis: Alpha fucosidase	Dried blood sample: 2 mL (1tubecontaining EDTA) Leukocyte analysis: 4 mL (2 tubes containing EDTA)
	Wolman–Cholesterol storage disease: Lysosomal acid lipase (LAL)	Dried blood sample: 2 mL (1tubecontaining EDTA)
	CLN 1: palmitoyl protein thioesterase	Dried blood sample: 2 mL (1tubecontaining EDTA)
	CLN 2: tripeptidyl peptidase I	Dried blood sample: 2 mL (1tubecontaining EDTA)
	Pompe Disease: Alpha glucosidase	Dried blood sample: 2 mL (1tubecontaining EDTA) Leukocyte analysis: 4 mL (2 tubes containing EDTA)
	Chitotriosidase	Dried blood sample: 2 mL (1 tube containing EDTA)
		Plasma: 2 mL blood (1 tube containing EDTA)

Ege University Children's Hospital Metabolic (Enzymology) Laboratory **Laboratory Analysis List- 2**		
Metabolite analysis	Lyso–Gb3	Dried blood sample: 2 mL (1 tube containing EDTA)
	Cystinosis- leukocyte cystine concentration	Leukocyte analysis: 4 mL (2 tubes containing EDTA)
Urine analysis	Iron 3 Chloride test Benedict's test (galactose) DNPH (urine ketoacids) test Sulphite test Para nitroaniline test (MMA)	10 mL spot urine
	Total glycosaminoglycans	4 mL spot urine
	tetrasaccharides	4 mL spot urine
	Sialic acid	4 mL spot urine
	Iodine	6 mL spot urine
	Total porphyrin Aminolevulinic acid (ALA) Porphobilinogen	8 mL, Aluminium foil wrapped (not affected by daylight)