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Original Article

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

Sebile Kılavuz[1], Fatma Derya Bulut[1], Deniz Kör[1], Berna Şeker Yılmaz[1], Sibel Başaran[2], Tunay Sarpel[2], Neslihan Önenli Mungan[1]

[1]*Çukurova University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Metabolism and Nutrition, Adana, Turkey*
[2]*Çukurova University Faculty of Medicine, Department of Physical Therapy and Rehabilitation, Adana, Turkey*

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

Address for Correspondence
Sebile Kılavuz MD, Çukurova University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Metabolism and Nutrition, Adana, Turkey
Phone: +90 505 707 64 98 E-mail: dr.skilavuz@gmail.com ORCID ID: orcid.org/0000-0002-7527-2620
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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

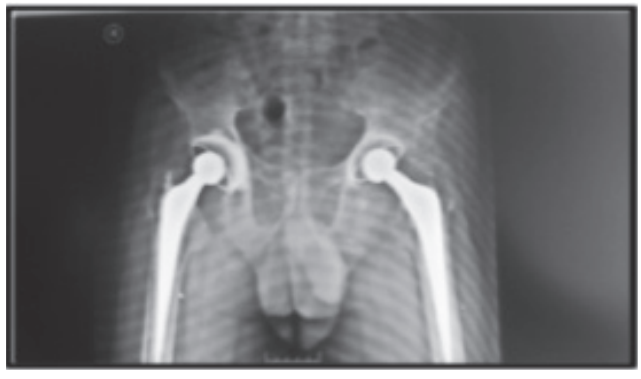

Figure 2. Total hip arthroplasty of family number 4

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.

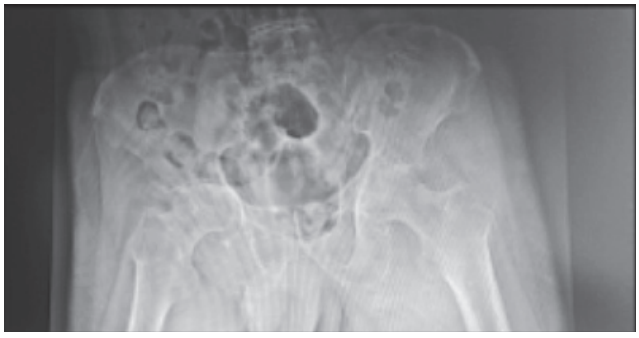

Figure 3. Ochronotic arthropathy of family number 4

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.

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

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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.