

The Pattern of Primary Hyperoxaluria Type I in Libyan Children at Tripoli Children Hospital, (1998-2018)

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ABSTRACT

Primary Hyperoxalurias are multiple autosomal recessive disorders involving the overproduction of oxalate. Primary Hyperoxaluria type (1) (PH1) is due to deficiency of alanine glyoxylate aminotransferase which is a hepatic peroxisomal enzyme, leading to excessive oxalate production, renal stone, nephrocalcinosis, and significant morbidity and mortality. The study aimed to describe the clinical and epidemiological patterns of PH1 in Libya. The study was descriptive case series type that included all children who were diagnosed as PH1 at Tripoli Children Hospital from May 1998 to September 2018. PH1 Diagnosis was established on the clinical presentation, positive family history of PH1, and elevated 24hr urinary oxalate, and the mutation of the AGXT gene. There were 60 children diagnosed as PH1, male composed of 57% of children. Their age at presentation ranged between 2 months and 10 years with a mean age at presentation of 3.5±3 years. The parents of 80% of these children had positive consanguinity and 91.7% had a positive family history. Seventy-five percent of patients were from South West (mountain area), 16(26.6%) of them were from Yefrin. Of these series 18(30%) presented with renal stones, and 18(30%) discovered by a family screening. The mean 24 hr. urinary oxalate excretion of these patients was 84.4mg %. Five different mutations were found, the most common mutation was c.731 T>C (P.Ile244thr) which was found in 38(84.4%) of children in this study. Interestingly, for those patients with a c.731T>C (p.Ile244thr) gene; 90% from South West (mountain area), and 3(10%) were from other areas with a high statistical significance (P=0.001). Of these children, there was 63.3% were from Yefrin. PH1 cases seen in Libyan children are especially clustered in those living in the Western (mountain area) mainly in Yefrin and Jadu. The most common age at presentation was under 5 years with positive family history. PH1 in Libyan children was most commonly expressed by the gene mutation c.731 T>C (P.Ile244thr), which is plausible given the limited genetic pooling caused by high consanguinity rates.

Keywords- Libya; Primary hyperoxaluria type 1; Nephrocalcinosis; Renal stones, Urinary oxalate; End-stage renal disease; Genetic renal disease.

INTRODUCTION

Primary Hyperoxalurias (PHs) are a group of autosomal recessive inborn errors of metabolism, which leads to the overproduction of endogenous oxalates from the liver, the excess oxalates deposit in many tissues, especially the kidneys, that potentially leads to renal damage and failure.¹ PH is divided into three types, (PH1), (PH2), and (PH3).² Of the three types, PH1 is the most challenging one, especially when it occurs in infancy.^{1,2} PH1 is further divided into three clinical subgroups. First, an infantile form with early renal insufficiency, which has the worst prognosis amongst all the subgroups. Second, a late-onset adult form, it is characterized by a better prognosis, and better renal function, and occasional stone passage. Third, usually presents with recurrent urolithiasis and progressive renal insufficiency, it is the most common subgroup. PH1 is due to either deficiency of alanine glyoxylate aminotransferase (AGT)

which is a hepatic peroxisomal enzyme, or by mistargeting of the enzyme to mitochondria instead of peroxisomes, leading to excessive oxalate production.³ The high amounts of oxalates exceed the renal filtration threshold, rendering high amounts in the body, which form calcium oxalates, resulting in crystalluria, nephrocalcinosis, and renal stone formation. Oxalates also precipitate in the eyes, heart, kidneys, and bones (systemic oxalosis), instigating significant morbidity and mortality.^{4,5} It accounts for 1-2% of cases of pediatric end-stage renal disease (ESRD) according to registries from Europe, the United States, and Japan.^{6,7} The prevalence appears more in countries in which consanguineous marriages are common.^{6,7}

About 20 to 50% of patients are suffering from chronic kidney disease (CKD), or ESRD when they are first diagnosed.^{2,6,8,9} However, Sometimes, the disease may not express any symptoms, these patients are usually discovered



by a family screening of an established patient, where have most likely the same disease severity as those who were detected through clinical suspicion, and hence family screening is important to identify cases who may possibly benefit from early initiation of treatment.¹⁰ The presentation varies from infantile nephrocalcinosis and failure to thrive as a result of renal impairment to recurrent or only occasional stone formation in adulthood.¹¹ About 20- 50% of patients have advanced Chronic Kidney disease (CKD) or even ESRD at the time of diagnosis.^{2,6,8} The median age at initial presentation is 4 to 7 years old, ranging from the early neonatal period to the sixth decade of life.^{9,11,12}

Mutation in AGXT, (the gene encoding AGT), resulted in PH1 AGXT composed of 11 exons and located on chromosome 2q36-q37.¹³ At least 178 pathogenic mutations have been described.¹⁴ The only known aspect of primary hyperoxaluria type one with a strong genotype-phenotype relation is responsiveness to Pyridoxine which occur in a patient with Gly170Arg and phe152Ile mutation.¹⁴⁻¹⁶ The Ile244Thr mutation is especially common in North Africa and Spain.^{17,18} About one-third of PH I patients are pyridoxine sensitive.¹⁹

The purpose of this study is the genes that caused PH1, the effect of consanguinity on the prevalence of the disease, the most common presentation in our region, and where the cases are especially clustered.

MATERIALS AND METHODS

This study was a retrospective case series type, which included all children who were diagnosed with PH1 at Tripoli Children Hospital (TCH) from May 1998 to September 2018. TCH is a referral hospital that provides medical services to children in the western part of Libya.

Data collected from the medical records included sex, date of birth (DOB), history of consanguinity, ethnicity (Arab/ Amazigh), family history of renal stone or hyperoxaluria, type of clinical presentation which include hematuria, abdominal pain, renal stones, evidence of urinary tract infection; ultrasound finding of renal stones or nephrocalcinosis. Sibling of patients also underwent screening by renal ultrasound and if there was found any positive findings, 24 hr urinary renal excretion was requested. The diagnosis of PH1 in the nephrology unit at TCH was based on the clinical presentation (renal stones or nephrocalcinosis), positive family history of PH1 and high 24-hour urinary oxalate, and presence of AGXT gene mutation. AGXT analysis done by Bioscientia Human Genetics (Ingelheim/Germany), Centgene AG (Rostock/ Germany) Institute of human genetics (Cologne Germany), and Rare Kidney Stone Consortium MYO clinic (USA). Hyperoxaluria was defined as urine oxalate > 0.5mmol/1.73m²/day.²⁰ In children who showed signs of severe renal failure and had low urine oxalate level, plasma oxalate level was obtained. All these children were under medical surveillance at the nephrology clinic. Clinical follow-up was done by normal renal function, follow-up with Chronic Kidney Disease (CKD), follow-up with End-Stage Renal Disease (ESRD), and follow-up children underwent hepato-renal transplantation.

Data coded then analyzed using IBM SPSS Statistics

for Windows, version 20 (IBM Corp., Armonk, N.Y., USA). Simple descriptive statistics as frequencies and percentage for categorical variables, and mean with the standard deviation (SD) for quantitative variables were performed. Chi-square test used for categorical variables with consideration of P-value < 0.05 as significant.

Informed consent was obtained from parents of all participants during their follow-up at the clinic and data confidentiality was maintained throughout the study and any resulting publication anonymously.

RESULTS

This study included 60 children diagnosed with PH1 at Tripoli Children Hospital during a period of 20 years (1998-2018). All these children were followed up in the nephrology unit at TCH. Boys were 57% of the cases, male to female ratio was 1.3:1. Their age ranged between 2 months and 10 years, with a mean age of 3.5±3 years. Two third (66.7%) of the cases were presented in the first four years of life. Most of the cases were originally from the mountain area, the vast majority of the cases (91.7%) reported positive family history of the disease, also 80% of the cases reported consanguinity marriage (Table 1).

Table 1: Socio-demographic characteristics of Libyan PH1 patients

Character	No.	%
Sex:		
Male	34	56.7
Female	26	43.3
Age (year):		
≤1	18	30
2-4	22	36.7
5-7	12	20
8-10	8	13.3
Ethnicity:		
Arab	30	50
Amazigh	30	50



Character	No.	%
<i>Residence:</i>		
Western mountain	45	75
Others	15	25
<i>Family history:</i>		
Yes	55	91.7
No	5	8.3
<i>Consanguinity:</i>		
Yes	48	80
No	12	20

Regarding place of residence, results showed that most of the cases were from Yafrin (26.7%), Jadu (15%), and El Orban (10%) (Figure 1).

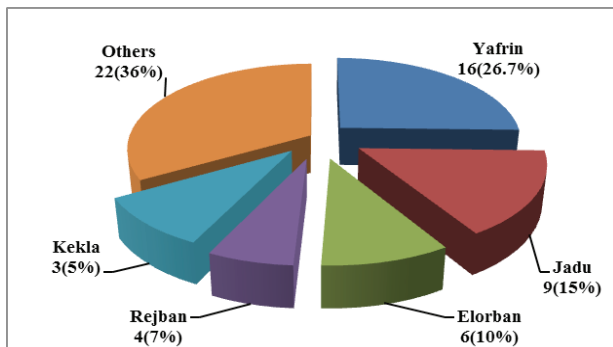


Figure 1: Distribution of children according to the place of residence

The results revealed that 30% of the cases presented with renal stones and 30% for family screening. Most of the cases were at a normal renal function at the time of presentation. Ultrasound reports at presentation showed 60% of cases had nephrocalcinosis and 30% had renal stones. Most of the cases (75%) were on regular follow-up with normal renal function, only 3 cases discontinued follow-up. ESRD was reported in 11.7% of the cases (Table 2).

Table 2: Clinical characteristics of Libyan PH1 patients

Character	No.	%
<i>Presentation:</i>		
Renal stone	18	30
Family screening	18	30
UTI	14	23.3
Hematuria	6	10
Nephrocalcinosis	4	6.7
<i>Renal function:</i>		
Normal	49	81.7
CKD	9	15
ESRD	2	3.3
<i>U/S finding:</i>		
Nephrocalcinosis	36	60
Renal stones	18	30
Normal	6	10
<i>Outcomes:</i>		
Follow up with normal renal function	45	75
CKD	2	3.3
ESRD	7	11.7
Hepatorenal transplantation	3	5
Lost to follow up	3	5

The AGXT mutation was done to 45 (75 %) cases. Homozygous mutation was seen in 61.7% of cases, compound heterozygous was seen in two siblings from the mountain area (Table 3).

Table 3: AGXT mutation analysis in Libyan PH1 patients

AGXT	No.	%
Homozygous	37	61.7
Heterozygous	6	10
Compound Heterozygous	2	3.3
Not done	15	25
Total	60	100



The results of mutations in the AGXT gene demonstrated that the most common gene mutation was c.731T>C (pIle.244thr) (84.4%), which mainly diagnosed among Amazigh children lived in the mountain area. There was a significant statistical difference between gene mutation type and area of residence ($P=0.001$), but no significant statistical difference between Arab and Amazigh patients ($P=0.1$) (Table 4, 5).

Table 4: AGXT gene mutation among Libyan PH1 patients according to residence area

AGXT	Mountain area No. (%)	Other areas No. (%)	Total No. (%)
c.731T>C(pIle.244thr)	33(91.7%)	5(55.6%)	38
c.466G>A(p.GLY156Arg)	0 (0%)	2(22.2%)	2
c.33dupC(p.Lys12GlnfsX156)	1(2.8%)	0(0%)	1
c.2_3delinsAT,c.907C>T)	2(5.5%)	0(0%)	2
c.1078C>Tp.(Arg360Trp)	0(0%)	2(22.2%)	2
Total	36 (100%)	9 (100%)	45

Table 5: Mutations in the AGXT gene according to ethnicity

AGXT	Amazigh No. (%)	Arab No. (%)	Total
c.731T>C(pIle.244thr)	22 (91.7%)	16(76.2%)	38
c.466G>A(p.GLY156Arg)	0 (0%)	2(9.5%)	2
c.33dupC(p.Lys12GlnfsX156)	0 (0%)	1(4.8%)	1
c.2_3delinsAT,c.907C>T)	2(8.3%)	0 (0%)	2
c.1078C>Tp.(Arg360Trp)	0 (0%)	2(9.5%)	2
Total	24 (100%)	21 (100%)	45

DISCUSSION

Primary Hyperoxalurias (PHs) are rare autosomal recessive disorders of the liver that result in the accumulation of toxic amounts of oxalates, which often cause kidney damage. There are three types of PH, (PH1, PH2, and PH3).²Our study comprised 60 children diagnosed with PH1. Consanguineous marriage is very common in North African and Middle Eastern cultures, as a result, it is not surprising to see a significant increase in prevalence compared to the lower prevalence seen in Europe, where PH1 was 1-3/ million, and an estimated incidence rate of 1:100,000 live births per year.^{8,21,22} Meanwhile, the prevalence increased up to 10% or higher in some North African (Tunisia) and Middle Eastern nations.^{1,2,23} In the present study consanguineous marriage was positive in 80% of the cases, also a vast

majority 91.7% demonstrated positive family history of the disease. This is not far from our previous study about PH1, where the consanguinity rate was 81.1%, and positive family history was seen in 83%.²⁴ In another study in Oman, the consanguinity rate was 100%, with each family having more than one affected child.²⁵ Mbarek et al. revealed a consanguinity rate of 75% in 57 patients from 40 different families.¹⁷ Another study of 26 patients from 20 families in Egypt reported 76.9% of consanguineous parents.²⁶

In the current study, the median age at presentation was 5 (range 2 months -10 years). This was lower than our 2018 study in which the median age was 9.9 (range 0.16 to 20 years). In a study conducted in Jordan, the median age at presentation was 3 years in comparison to 6 and 13 years in the Netherlands and Japan respectively.^{1,8,27} However, Mbarek et al. reported that the median age at presentation for patients carrying the 33-34insC mutation in Tunisian children was 3 years (range 5 months–61 years). On the other hand, children carrying the 1244T mutation were older, with a median age of 13 years (range 3 months–38 years).¹⁷

The diagnosis of PH1 was established in about 10% of Libyan children with end-stage renal disease (ESRD)²⁴, similar to that seen in a Kuwaiti study.²⁸ Whereas in Europe, the United States, and Japan, the diagnosis of PH1 accounted for 1-2% of cases of ESRD.^{1,23} In this study, the most common presentation was renal stones seen in 30% of the cases, while the percentage of cases presented with ESRD was 11.7%. In Soliman et al. study carried in Egypt, the most common presentation was ESRD in 65.4% of the cases.²⁶ In a study performed on some Jordanian children, the most common presentation was hematuria (22.8%).²⁷

In this study, the high rate of homozygous mutations (61.6%) was strong reminiscent of the confounder effect of the high consanguinity rate (80%), this per the previous study, where the homozygous mutation rate was (78.1%), and consanguinity rate was (81.1%).²⁴ Of the 5 gene mutations we identified, the most common gene mutation was c.731T>C(pIle.244thr) (84.4%), which was mainly detected among Amazigh children from the West Mountain area (91.7%), the most common gene identified, and the most affected area were both analogous to our previous study.²⁴ In a Tunisian study, the allele frequency of 1244T was noted to be predominant (68%), the 33_34insC mutation came second at 32%, amongst other less frequent gene mutations.¹⁷ In contrast, a large Hoppert al. cohort study performed in the United States on 247 patients with PH1, the genotype-phenotype correlations at the genic, and allelic levels were evaluated. This cohort study displayed that the most common two AGXT variants accounted for 47.8% of PH1 mutant alleles (p.G170R and c.33dupC 15.5%).²⁹

Limitations

The limitations that were encountered in this study are the lack of investigations for PH1 in Libya (24hr urinary oxalate collection and AGXT gene mutation analysis). As a consequence, the samples were sent to Germany and United States laboratories. Furthermore, the feasibility and financial burden hindered the collection of a larger sample size, which in turn may have an impact on the reliability of our data in future analysis.



CONCLUSION

PH1 cases in Libya are especially clustered in the West Mountain area. The most common gene mutation identified was c.731T>C (p.Ile244Thr), this might be helpful in future screening, counseling, and management of PH1 in Libya. Around two-thirds of the cases presented in the first four years of life. Renal stones were the most common presentation exhibited in one-third of the cases.

RECOMMENDATIONS

To decrease the incidence of the disease in our country we recommend: the increase of awareness and knowledge of the families regarding the role of consanguineous marriage in the inheritance of this disorder, genetic testing, along with family counseling and premarital screening program especially in places where PH1 is prevalent. Since for the time being the only accessible drug for the treatment of PH1 is Pyridoxine, further studies regarding the efficacy of pyridoxine on PH1 expressed particularly by the genes is recommended.

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