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Unusual Outcome of Primary Hyperoxaluria Type 1 in Adult Patients with 33_34InsC Mutation

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Abstract: ***Primary hyperoxaluria type 1 is a rare autosomal recessive inborn error based on absence, deficiency or mislocalization of the liver-specific peroxisomal enzyme alanine:glyoxylate aminotransferase (AGXT). Some mutations arevery rarely described in the adult patients as the 33-34InsC mutation, known as responsible for a terrible severe clinical feature that can lead to early death occurring the childhood period. 68 adult patients have been diagnosed for c.33_34InsC, p.G170R, p.I244T, p.F152I, p.G190R, p.W108R and p.976delG mutations in AGXT, as first diagnosed line. We described eight adult patients with a 33-34InsC mutation in homozygote state with a median age of 50±12.51 years old. Five of them reached the end stage renal disease (ESRD) at the median age of 55.8±12.31 years old. The three other patients preserved until now a normal renal function. We observed a mild and unusual clinical course of primary hyperoxaluria toward ESRD with only spontaneous elimination of urolithiasis for the majority of the patients. Primary hyperoxaluria type 1 is a heterogeneous disease and a clinical course of patients with severe PH1 mutations is not dictated primarily by its genotype. The implication of other genetic and/or environmental factors can play a crucial role in determining the ultimate phenotype.***

Keywords: Primary hyperoxaluria type 1, Tunisian population, Urolithiasis, 33-34InsC mutation.

1. Background

Primary hyperoxaluria type 1 (PH1, OMIM 259900) is a rare autosomal recessive inborn error of the glyoxylate metabolism that is caused by an absence, deficiency or mislocalization of the liver-specific peroxisomal enzyme alanine:glyoxylate aminotransferase (AGXT). A massive elevation of urinary oxalate excretion results in recurrent kidney stones and/or progressive nephrocalcinosis and often to early end-stage renal disease (ESRD) [1]. As the disease progresses,systemic oxalosis, even in the stage of mild renal insufficiency, plasma oxalate levels begin to increase and calcium oxalate deposition occurs in various tissues. This engenders serious complications as well as a fatal outcome. [2] Conservative measures should be initiated as soon as the investigations are completed and the renal function is maintained. Once the ESRD is established, pyridoxine is the only specific treatment that should be prescribed [2]. Only combined liver-kidney transplantation can cure the defect whereas isolated kidney transplantation will definitively lead to the disease recurrence [3].

PH1 is very heterogeneous disease that can affect individuals regardless of their age. The presentation varies from infantile nephrocalcinosis and failure to thrive as a result of renal impairment to recurrent or only occasional stone formation [4]. Although patients affected during adulthood often have a history of only sporadic stone disease, over 50% of them are present with ESRD at the time of diagnosis [5, 6].
At least, 178 pathological mutations in *AGXT*gene have been documented [7]. Some mutations were very rarely described in the adult patients as the 33-34InsC mutation known responsible for a very severe clinical feature and early death in the childhood age [8]. Severity is explained by the fact that this insertion leads to a truncated protein without catalytic activity [9].

In this report, we describe adult patients with a mild and unusual clinical course of primary hyperoxaluria toward ESRD. The diagnosis of PH1 was confirmed with mutational analysis of the *AGXT* gene, which showed that the patient was homozygous for 33_34InsC mutation.

2. Patients and Methods

2.1 Subjects

Since 2007, 68 adult patients have been diagnosed with PH1 and different stage of chronic renal failure. We consider in our study a highly selected cohort of patients and not a population-based sample. They were addressed by different nephrology departments of Tunisia. The median age of diagnosed patients was 49.75±11.44 years old with sex-ratio of 1. No liver biopsy was carried out on our patients because diagnosis was confirmed by genetic testing.

In order to decrease supersaturation with oxalate, most cases undertake hyperhydration and pyridoxine (vitamin B6) supplementation.

2.2 Molecular Approach

After obtaining patients' consent, genomic DNA was isolated from peripheral blood leucocytes, as described previously [10].
Six mutations, that described as recurring frequently and that form the basis of DNA screening panels in Tunisia and European countries [11, 12], were tested as first diagnosed

line. The c.33_34InsC (p.Lys12GlnfsX156), c.508G>A (p.G170R), c.731T>C (p.I244T) and c.454T>A (p.F152I) mutations in *AGXT* were analyzed by amplification of genomic DNA and restriction enzyme digestion using previously documented [8] primers and conditions. PCR detecting 74pb duplication in exon 1, was used to distinguish the Ma and Mi alleles, andPCR/ restriction enzyme test using MwoI was used to genotype for c.33_34InsC [8].

2.3 Ethical Approval

The adult patients provide informed consent to the diagnostic and therapeutic procedures involved, in agreement with the guidelines approved by our institutional clinical research ethics committee.

3. Results

In this study, eight adult patients with a sex-ratio of 1.6 (three women and five men) were identified with 33-34InsC mutation. All of them were homozygotes for the mutation and have the major alleles (M/M). Five patients were index cases,however, the three others were diagnosed during familial investigations.

The detection of PH1 in our cohort was dilated by unspecific symptoms of nephrolithiasis. All of them were diagnosed in the adulthood and the median age was 50 years (range 36 to 73 years). Further, the median age of onset of ESRD was 55.8 years (range 43 to 73 years).

Case 1:A 64-year-old female presented at the department of nephrology with bilateral flank pain and signs of advanced chronic renal failure (CRF) since the beginning of 2006. The patient has the history of hypertension and gout. It was her first clinical consultation, and the diagnostic workup revealed a normal kidneys size, hyperechogenic kidney parenchyma, unique small stone in the calyces of both kidneys and suspected nephrocalcinosis. In addition, she had a small calculus in the bladder and a history of elimination of an only one calculus a few years ago. Puncture renal biopsy demonstrates the presence of intratubular oxalate crystals. There were crystals of oxalate and uric acid observed at the level of the urinary sediment, without proteinuria and microscopic hematuria.

Case 2: The 50 years-old man, was addressed in 2012 for renal failure. The patient was already in ESRD with 2175 µl/L of creatinemia. He has a personal and a family history of urolithiasis. His last lithiasis was eliminated by extracorporeal shock wave lithotripsy in 2001. The genetic screenings in family confirm the diagnosis for his father (case 3).

Case 3: It was a man of 73 years-old, suffering CRF and having a history of spontaneous elimination of lithiasis. Interestingly he preserved until 70 years-old a normal renal function. At his last following up the patient reached the ESRD.

Case 4: 43 years-old man, with history of recidivate urolithiasis since the age of 9 and with spontaneous emission of calculi. CRF was installed in 2012 as evolution of an obstructive renal failure, after an elimination of an obstructive urolithiasis by extracorporeal lithotripsy and the setting of a ureteral JJ stent. The patient has also a family history of lithiasis. His brother, 40 years-old, (**case 5**) suffered also from urolithiasis eliminated spontaneously, but preserved normal renal function.

Case 6: In the outset of 2012, a 49-years-old female was presented, for a first consultation in nephrology department, with bilateral flank pain and signs of advanced CRF with 0.6ml/minute of clearance of createnuria. She has the family history of urolithiasis, in fact her niece is died in hemodialysis stage at the age of 12 year-old. The renal echography showed non differentiated small kidneys. Cristalluria identified the type IC calculi.

Case 7: A 36-years-old female was addressed by the urology department for recidivist urolithiasis. The first symptoms appeared at the age of 7. The analysis of the calculi proved the presence of the type Ia calculi with 98% of whewellite. Cristalluria identified the presence of monohydrate calculi and oxalate crystals. The patient belongs to a very consanguineous family who has a history of urolithiasis. Her brother operated also for urolithiasis in one occasion. Her aunt, in addition, operated in advanced age of urolithiasis. In the last follow up, the patient preserved a normal renal function and a normal value of oxaluria.

Case 8: A 45-years-old man, he is the brother of case 7 and was enrolled in the familial investigation. First symptoms were essentially urolithiasis, appeared at the age of 45 and because of which he has a chirurgical intervention during 2013. In the last follow up in 2015, the patient preserved a normal renal function and a normal value of oxaluria.

Clinically, PH1 has variable presentation and patients presented several symptoms at the time of diagnosis (Table I). In fact, some signs corresponded mainly to manifestations of CRF, but other signs were not specific such as anemia, diarrhea, vomiting and general alteration.

The history of urolithiasis was present in all the eight patients with detected 33-34insC and was the main cause of diagnosis in two of them. Actually, two patients have their first renal colic until the pediatric age, (7 and 9 years). 3/8 patients have spontaneous elimination of calculi; while5/8 of them suffered recidivist nephrolithiasis, eliminated using extracorporeal shock wave lithotripsy.

Since the diagnosis 62.5% (5/8) of our patients reached the ESRD, whereas 37.5% (3/8) preserved until now a normal renal function with a median age of 40.3years (range 36 to 45 years). To summarize, the clinical symptoms and the index cases' data were presented in the Table I.

Table 1: Clinical symptoms in affected index cases with 33-34InsC mutation

Patients	1	2	3	4	5
Age	64	50	43	50	36
Sex	F	M	M	F	F
Geographic origin	Libya	Kasserine	Kasserine	Kairouan	Kasserine
Age of First symptoms (years)	64	49	32	48	7
First symptoms	Bilateral flank pain / gout	Urolithiasis/ CRF	Recidivist urolithiasis	Bilateral flank pain	Recidivist urolithiasis
Renal ecography	Hyperechogenic kidney parenchyma	Nephrocalcinosis	Non differentiated kidney/Bilateral Lithiasis	Non differentiated kidney	Normal
kidneys size	Normal	Normal	Normal	Small kidneys	Normal
Oxalemia (7-66 µmol/L)	ND	ND	30µmol/L	ND	25 µmol/L
Renal biopsy	Intratubular oxalate crystals	ND	ND	ND	ND
Cristalluria	ND	ND	ND	type IC calculi (whewellite)	a calculi with 98% of whewellite
Renal Function	ESRD	ESRD	CRF	ESRD	Normal
Extra-renal manifestation	Hypertension / gout	Hypertensive retinopathy/ Type 2 Diabetes / Hypertension	No	No	No
Diagnosed mutation	Homozygous 33-34InsC	Homozygous 33-34InsC	Homozygous 33-34InsC	Homozygous 33-34InsC	Homozygous 33-34InsC

M male; ***F*** female; ***ND*** not done; ***ESRD*** end stage renal disease; ***CRF*** chronic renal failure

4. Discussion

Primary hyperoxaluria type 1, the most common form of primary hyperoxaluria, is a very rare inherited disease. The highest rates were found in Mediterranean countries especially in Tunisia [13, 14]. Indeed, PH1 is responsible for more than 13% of ESRD in children in Tunisia versus 10%, 0.3% and 0.7% in Kuwait, Europe and North America, respectively [13,15]. Actually, PH1 is most prevalent in Arabic countries due to genetic make-up and a higher rate of consanguineous marriages [16].

PH1 is very heterogeneous disease that can cover several presentations. The infantile form is characterized by early nephrocalcinosis and kidney failure, usually one half of patients experience ESRD at the time of diagnosis and 80% develop ESRD by the age of 3 years. Recurrent urolithiasis and progressive renal failure are mainly described in childhood or adolescence onset of PH1. However, the adult form is typified with a late onset disease and occasional stone passage. Furthermore, the PH1 diagnosis can be made by post-transplantation recurrence and pre-symptomatic subjects with a family history of PH1 [4].

Molecular genetic testing has been proved to be an efficient alternative and non-invasive approach, as was liver biopsy, to confirm the definitive diagnosis of PH1. It was already demonstratedthat the four common mutational changes c.508 G>A (p.G170R), c.33_34InsC (p.Lys12GlnfsX156), c.731 T>C (p.I244T, Maghrebian) and c.454T>A (p.F152I) are the most recurrent mutations with a high frequency in Caucasians populations, respectively with 40, 12, 9 and 7% of disease alleles [17, 18].

The principal mutations described in adult patients around the world, were G170R, F152I and I244T mainly in Canary Island and Tunisia [19, 11]. However, the 33_34 InsC was mainly descried in pediatric cases (12%) and it was very rarely reported in late onset, particularly in the homozygote's states [1, 19, 12, 20]. This micro-insertion was considered as the most common PH1 mutation on the major allele (31%) of *AGXT* [8]. It has been reported that homozygouswould be expected to have no immune-reactive protein and no catalytic activity [9]. It is also associated with a very sever disease.

33_34InsC mutation would appear as a pan ethnic. It was first described in the Italian population [21]. Given the historical migratory/invasive flows that took place in North Africa, we can suggest that this variation is associated with a specific population or ethnic group [22]. Indeed, 57% of our patients are originated from the city of Kasserine, situated in West-Central Tunisia, known by Roman invasion [23]. We hypothesize that this variation, as many other mutations [24], might have been introduced in Tunisia during the Roman settlement up to the 5th century AC.

In this report, we described 11.26% (8/71) of our adult patients bearing the 33_34InsC mutation in homozygote state, and in our knowledge, it is the largest number of patients carrying this mutation and having the same ethnicity. We have formerly reported that the 33_34InsC mutation, is the second mutation detected in our patients with alleles frequency of 32% [11], more elevated than other reports (12 to 13%) [12, 8] and it was associated with a very severe clinical form in children patients [11].

Weirdly, the clinical manifestations were entirely different between children and adults patients. In fact, 62.5% of children carrying this mutation died of their disease with a median age of 3 years [11]. Nevertheless, we noted the absence of the expected severity attributed to 33_34InsC

mutation in adults'. Indeed, all of our patients have a slowly development of the clinical symptoms of the PH1 characterized by urolithiasis. Only five of them reached ESRD at the median age of 54±8.71 years-old because they were diagnosed later after the onset of renal insufficiency and have no medical follow-up. Curiously, three of our adult patients preserved a normal renal function until the age of 40 without B6 treatment. Only two of our patients have initial symptoms as recidivist lithiasis from the age of 9 (case 4) and 7 (case 7). The case 7 preserved a normal renal function; however case 4 developed CRF at 43 years-old.

Strangely enough, a marked intra-familial clinical heterogeneity was noted, although the patients harbor the same AGXT mutations. Some are present with early and severe clinical manifestations, whereas others may be asymptomatic for long time. In fact, the case 6, were asymptomatic for many years and even with a near-normal urinary oxalate excretion, has first symptoms' disease in the age of 49. However, her niece died at the age of 12 years, after a history of urolithiasis and kidney disease. As well as, the case 3 preserved until the age of 70 a normal renal function; however, his sun (case2) started the CRF much earlier at the age of 50.

This variation in the age of diagnosis and clinical symptoms can be related to many factors such as interactions with other genes, coding for other possibly glyoxylate-utilizing enzymes, and/or with environmental factors. Many hypotheses were suggested to explain the effect of the diet. A restriction of the intake of oxalate-rich food, as chocolate, spinach, walnuts, black tea, etc; was suggested in PH patients because of their lower intestinal oxalate absorption,compared to normal subjects [25]

However, it was reported that fiber intake leads to a change in bowel transit that diminishes the absorption of both calcium and oxalate, thus reduces the incidence of calcium lithiasis. For that reason, fiber intake is recommended for patients with recurrent lithiasis [26, 27].Nevertheless, there is not enough scientific evidence to corroborate the benefits of this measure. In spite of the precocity of primary symptoms in some of our patients, the stage of CRF was delayed under the age of 40. We suggested that the diet can be seriously implicated in this feature. Interestingly, the diet costumers in central and West-central regions are based on a rich fiber intake in their food (eg apples,barley, whole wheat bread, spices…)and nutriment rich in vit B12 as fatty fish, lamb, offal, cereal, bananas…

5. Conclusion

The 33_34InsC mutation causing primary hyperoxaluria type 1 is a severe mutation that seems to provide a high morbidity and ruthless infantile PH1. We report that 33_34insC can be present in adults' forms and mild clinical features, suggesting the other factors implication in this slowly progress.

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Conflicts of Interest

The authors declare no conflict of interest.

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