Original Article

Spectrum of Organic Aciduria Diseases in Tunisia: A 35-year Retrospective Study

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Abstract

Background: Organic aciduria diseases (OADs) occur worldwide, with differences in prevalence and patterns between populations.

Objectives: To describe the spectrum of OADs identified in Tunisia over a 35-years period.

Materials and Methods: This retrospective study included patients who were diagnosed with OADs between 1987 and 2022 in the Laboratory of Biochemistry, Rabta Hospital, Tunisia. Organic acids were analyzed using gas chromatography–mass spectrometry.

Results: A total of 30,670 urine samples were analyzed for OADs, of which 471 were positive for OADs. The estimated incidence of OADs in Tunisia was 6.78 per 100,000 live births. Methylmalonic ($n = 146$) and propionic ($n = 90$) acidurias were the most common OADs (estimated incidence: 2.10 and 1.30 per 100,000 live births, respectively). There were 54 cases of L-2-hydroxyglutatric acidurias and 30 cases of pyroglutamic acidurias, which makes it one of the highest in the world. The main clinical features were hypotonia (65%) and feeding difficulties (41%). Age at diagnosis was highly variable, ranging from 1 day to 49 years. Only 27% of the patients were diagnosed within the first month of life. The prevalence of OADs was highest in the Center-East and Southeast regions.

Conclusions: In Tunisia, OADs are relatively frequent, but there are shortcomings regarding the diagnosis of these disorders. The frequency and health/social impact of these disorders warrant the need for implementing newborn screening programs and suitable patient management.

Keywords: Incidence, inborn errors metabolism, inherited errors of amino acid metabolism, epidemiology, diagnosis, organic acidurias, Tunisia

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INTRODUCTION

Organic aciduria diseases (OADs) are a heterogeneous group of inborn errors of metabolism, classified as intoxication-type metabolic diseases (E-IMDs).[1] These hereditary disorders are mainly transmitted in an autosomal recessive mode. OADs are due to defects in intermediary metabolic pathways of carbohydrates, amino acids, and fatty acids.[2] Enzymatic defects in specific pathways result

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in an accumulation of organic acids in tissues and their subsequent excretion in urine.[3] Patients with OAD are at increased risk of disability, impaired quality of life, and reduced life expectancy. Together with aminoacidopathies, OADs are considered the most frequent inherited metabolic disorders among severely ill children.[4-6] The incidence and patterns of OADs vary between populations.[7] Their diagnosis requires the identification of abnormal patterns of organic acids in biological fluids, especially in urine, by gas chromatography–mass spectrometry (GC-MS).[8] The GC-MS procedure requires expensive equipment and qualified professionals, and thus, in low-income countries, the diagnosis of OADs is generally available only in few regional reference centers.[9-11] Moreover, the diagnosis and management strategies of OAD vary between countries, resulting in significant disparity in patient outcomes.

There is limited literature regarding the characteristics of OADs in Tunisia. Hadj-Taieb *et al*.,[11] in their description regarding the epidemiological and clinical aspects of OADs in Tunisia, reported a high incidence of the disorders with a predominance of methylmalonic aciduria (MMA) and propionic aciduria (PA). However, the data are now dated, and thus there is need for more recent data. Accordingly, the current study is a comprehensive update of OAD characteristics in Tunisia, covering a large series of patients diagnosed over a 35-year period. The findings would contribute to raising the knowledge and awareness of OADs among health professionals and policymakers in Tunisia, and consequently, in implementing accurate strategies for their diagnosis and management.

MATERIALS AND METHODS

Study design, setting, and population

This retrospective study included patients who were diagnosed with OAD between 1987 and 2022 in the Laboratory of Biochemistry at Rabta Hospital, Tunis, Tunisia. The laboratory is the only site in Tunisia that performs analysis of organic acids. The protocol of the study was approved by the Ethics Committee of Rabta Hospital.

Over the 35 years, patients with clinical findings suggestive of E-IMDs, such as lethargy, hypotonia, acute encephalopathy, and sudden infant death of unknown cause or biochemical abnormalities including acidosis, ketosis, hyperammonemia, and hypoglycemia, were screened on request. The samples of patients were received from pediatrics, neonatology, and pediatric neurology departments in public and private health facilities throughout the country. Blood and fresh random urine samples were referred to the laboratory along with a pre-established data sheet form providing data relating to the patient, including demographic data, history of personal and family health, detailed description of symptoms and clinical signs, and the results of the first-line biological assessment. The diagnosis of OAD was established based on a urine organic acids chromatogram profile that is specific for OAD.

METHODS

Basic metabolic investigations were performed using specific methods. These included serum glucose, electrolytes, creatinine, liver function tests, lactate, ammonia, arterial blood gases, and urine ketone bodies. The profile of organic acids was analyzed by GC–MS, as described by Sweetman.[12] Briefly, urine sample with volume corresponding to 1 mmol of creatinine was first acidified to pH 1.5–2 and mixed with two internal standards (butyric acid and heptadecanoic acid) and then extracted three times with ethyl acetate. Derivatization was performed with bis (trimethylsilyl) trifluoroacetamide + 1% trimethylchlorosilane and pyridine at 80°C for 45 min. Trimethylsilyl derivatives were analyzed using an Agilent 5975/7890A GC–MS (Agilent Technologies Inc. USA) with EI source, operated under the control of Agilent MSD ChemStation software. Chromatographic separation was performed by a capillary column (Agilent HP-Ultra2; 25 m × 0.20 mm ID, with a 0.33-μm film thickness of 5% phenylmethyl silicone) and helium as carrier gas. The GC-MS temperature program was as follows: injector 250°C, column 90°C to 280°C with an increment of 5°C per minute, and the run time of each analysis was 52 min. Estimated incidence ($1/E$) of OADs in Tunisia was calculated using the following formula: $E = (N/M)+f \times(N/M)^{1/2}$, where (M) is the number of patients with OAD diagnosed during the study period, (N) is the number of births during the study period and (f) is the coefficient of consanguinity in Tunisia (0.0129). Demographic data including the annual number of live births during the study period were obtained from the National Institute of Statistics of Tunisia (http://www.ins.tn/fr/statistiques).

RESULTS

In the 35-year period, 30,670 patients with symptoms suggestive of OAD or asymptomatic patients issued from affected families had their urine specimens analyzed for the pattern of organic acids. Among them, 471 patients were diagnosed with an OAD: 274 males (58.2%) and 197 females (41.8%). During this period, 6,949,900 live births had occurred in Tunisia; therefore, the estimated incidence of OADs in Tunisia was 1:14,757 live births (6.78

per 100,000 live births). Sixteen different OADs were identified, with MMA (30.9%) and PA (19.1%) being the most common. Age at the time of diagnosis was highly varied between OADs as well for each OAD, ranging from 1 day to 49 years [mean ± SD: 36 ± 76 months] [Table 1].

A diagnosis was made after the age of 3 months of life in 60% of the patients, between 1 and 3 months of life in 11%, and within the first month of life in 27%, with large disparities between OADs [Figure 1]. In affected families, 32% of the OADs, particularly methylmalonic, propionic, isovaleric, and pyroglutamic acidurias (PGA), were diagnosed within the first days of life in asymptomatic or pauci-symptomatic patients. Around three-quarters of patients (77.6%) were born from consanguineous marriages, with first-degree consanguinity noted in 36%. Patients with OADs were from all regions of Tunisia, but the prevalence was highest in the Center-East and Southeast regions. Specifically, MMA was most prevalent in the northern half of Tunisia, propionic and L-2-hydroxyglutatric acidurias (2-HGA) in the Southeast, and pyroglutamic and isovaleric acidurias (IVA) in the center.

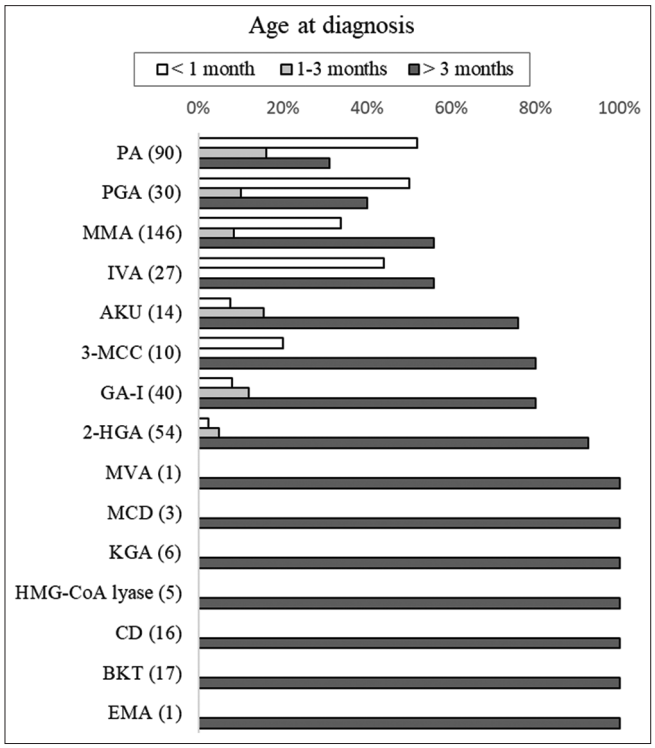

Figure 1: Age at diagnosis in Tunisian patients with organic aciduria diseases. The number between parentheses refers to the number of patients diagnosed; 2-HGA – 2-hydroxyglutatric aciduria; 3-MCC – 3-methylcrotonyl-CoA carboxylase deficiency; 3-methylglutaconic aciduria; AKU – Alkaptonuria; BKT – β-ketothiolase deficiency; CD – Canavan disease; EMA – Ethylmalonic aciduria; GA-1 – Glutaric aciduria type 1; HMG-CoA lyase – 3-hydroxy-3-methylglutaryl-CoA lyase deficiency; IVA – Isovaleric aciduria; KGA – α-ketoglutaric aciduria; MCD – Multiple carboxylase deficiency; MMA – Methylmalonic aciduria; MVA – Mevalonic aciduria; PA – Propionic aciduria; PGA – Pyroglutamic aciduri

Clinical features of OADs are dominated by neurological symptoms, especially hypotonia, and metabolic acidosis was the main laboratory anomaly. The main clinical and biochemical features of patients with OADs are depicted in Figure 2. After diagnosis, patients were treated with diet, medicine, and rehabilitation. The care was different according to the type of OAD and its quality depended on the skills and resources of the medical team in charge of the patient, as well as the socio-economic conditions of the family. Few patients benefited from adequate care with good respect for the diet and accurate clinical and biochemical monitoring, resulting in clinical improvement in few patients. The management was inadequate for most patients due to difficulties in adhering to a low-protein diet and obtaining costly special preparations. The progress of the disease and outcome remained unknown for most patients.

DISCUSSION

This study is a comprehensive report describing the incidence, pattern, and main characteristics of primary organic acid metabolism disorders in high-risk Tunisian patients over a 35-year period. During the investigation period, through clinically guided screening, 471 patients were diagnosed with OADs versus 837 patients with aminoacidopathies, accounting for 36% of the small-molecule E-IMD group. The findings of this study estimated the overall incidence of OAD of about 1:15,000 live births, which is in line with the average incidence worldwide. OAD incidence has been estimated to be

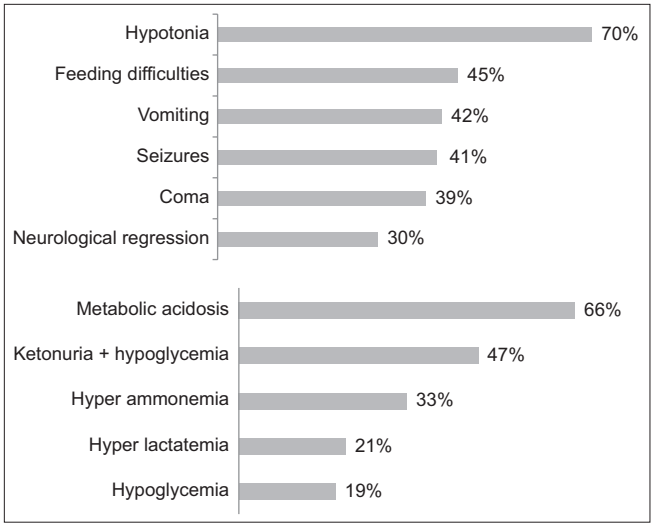

Figure 2: Main clinical features (a) and laboratory findings (b) in Tunisian patients with organic aciduria diseases

Table 1: Profile and estimated incidence of organic aciduria diseases detected from 1987 and 2022 in the Tunisian population (*N*=471)

OA disease type	Number of patients (percentage of OAs)	Age at diagnosis (months) median (5th–95th percentile)	Estimated incidence	
			Per total live births	Per 100,000 live births
MMA	146 (30.9)	5 (0.10–120)	1:47,605	2.100
PA	90 (19.1)	1 (0.10–24)	1:77,225	1.294
2-HGA	54 (11.5)	118 (4–384)	1:128,706	0.776
GA-1	40 (8.5)	11 (0.9–108)	1:173,753	0.575
PGA	30 (6.5)	2 (0.2–84)	1:231,670	0.431
IVA	27 (5.5)	19 (0.2–120)	1:257,410	0.388
BKT	17 (3.6)	16 (5–120)	1:408,826	0.244
CD	16 (3.4)	14 (6–76)	1:434,377	0.230
AKU	14 (2.9)	48 (0.2–588)	1:496,431	0.201
MGA	11 (2.3)	34 (0.0–48)	1:631,819	0.158
3-MCC	10 (2.1)	34 (0.1–180)	1:695,001	0.143
KGA	6 (1.3)	3 (0.6–36)	1:1,158,331	0.086
HMG-CoA lyase deficiency	5 (1.1)	21 (4–48)	1:1,389,995	0.071
MCD (glutaric 2)	3 (0.6)	0.6 (0.4–6)	1:2,316,653	0.043
EMA	1 (0.2)	0.6	1:6,949,934	0.015
MVA	1 (0.2)	7	1:6,949,934	0.015

OA – Organic aciduria; 2-HGA – 2-hydroxydglutatric aciduria; 3-MCC – 3-methylcrotonyl-CoA carboxylase deficiency; MMA – Methylmalonic aciduria; PA – Propionic aciduria; GA-1 – Glutaric aciduria type 1; PGA – Pyroglutamic aciduria; IVA – Isovaleric aciduria; BKT – β-ketothiolase deficiency; CD – Canavan disease; AKU – Alkaptonuria; MGA – 3-methylglutaconic aciduria; KGA – α-ketoglutaric aciduria; HMG-CoA lyase – 3-hydroxy3-methylglutaryl-CoA lyase; MCD – Multiple carboxylase deficiency; EMA – Ethylmalonic aciduria; MVA – Mevalonic acid

about 1:31,000 in Korea,[7] 1:22,000 in Japan,[7] 1:12,000 in Italy,[2] 1:10,000 in Germany,[7] and 1:6565 in Singapore.[13] Scarce data are available on OAD incidence in Africa and the Arab world, except in the Gulf region. The very high incidence of 1:5500 live births reported in a previous study from Tunisia[11] appears to be an overestimation, as the incidence assumes that 40,000 live births occur per year in Tunisia, whereas the correct number of live births is around 180,000.

Most previous studies have reported individual OAD incidence rather than the overall incidence and especially described the pattern of OADs in different populations. In Tunisia, the pattern is characterized by a predominance of MMA and PA. These two disorders account for half of OADs detected in Tunisia. MMA is the most common OAD in different populations, ranging from 30% to >50% of detected OADs, and even about 60% in two Chinese series.[14,15] The estimated incidence of 1:47,600 in Tunisia is in line with the average worldwide incidence of 1:50,000.[16,17] It is lower than the incidence in the series from Kuwait (1:19,809)[18] and China (1:20,000),[19] but higher than that reported in Germany (1:159,199).[18]

In the absence of genetic analyses, we were unable to distinguish the forms of MMA in Tunisia. However, at least 12 patients had positively responded to vitamin B12 therapy. PA is the second most frequent OAD in Tunisia, accounting for a fifth of the OADs detected. The two OADs are the most detected in different populations with a predominance of MMA. However, PA is more frequent than MMA in Colombia,[20] Oman,[21] and Bahrain.[22] The incidence of PA varies widely between populations, with a worldwide incidence of 1:100,000 to 1:150,000 live births.[17,23] The incidence of 1:77,200 in Tunisia is lower than that in the Arabian Gulf (1:20,000 to 1:45,000).[24,25] The highest incidence has been reported in Greenlandic Innuits (1:1000)[26] and in two Saudi tribes (1:2000 and 1:5000).[27] This disorder is less frequent in the United States (1:105,000 to 1:130,000)[28] and Europe (1:160,000 to 1:250,000).[29,30]

In Tunisia, 2-HGA and PGA account for 11.5% and 6.3% of all OADs, respectively. The frequency of both the disorders is higher in Tunisia compared with the other regions of the world. The current study detected 54 cases of 2-HGA and 30 cases of PGA, while only approximately 300 cases of 2-HGA and 80 cases of PGA have been reported worldwide.[31,32] The estimated incidences of 1:128,700 for 2-HGA and of 1:231,500 for PGA in our series are remarkably higher than the worldwide incidence estimates of 1:1,000,000.[33] Most of the affected patients originated from the same geographic region (Southeast for 2-HGA and the center for PGA), which confirms a founder effect for these two disorders. Worldwide, most of the reported cases were from Saudi Arabia,[24] Brazil,[34] and Canada[35] for 2-HGA, and from France and Germany for PGA.[36,37]

Glutaric-1 aciduria, IVA, beta ketothiolase and 3-methylglutaconic aciduria, Canavan disease, alkaptonuria, and 3-methylcrotonyl-CoA carboxylase deficiency account for 2% to 8% of all OADs in Tunisia. These incidences, estimated through selective screening, fall within the average

incidence in the world. Glutaric-1 aciduria is particularly frequent in Canada[35] and Brazil,[34] IVA is frequent in Iran[38] and Oman,[21] and 3-methylglutaconic aciduria is frequent in Israel (in the Iraqi Jewish population).[39] Other OADs, including α-ketoglutaric, ethylmalonic, and mevalonic acidurias, 3-hydroxy 3-methylglutaryl-CoA lyase deficiency, and multiple carboxylase deficiency, account for 0.2% to 1.3% of all OADs, and thus are rare in Tunisia. Table 2 shows the pattern of OADs detected in different regions of the world.

The incidence of OADs described in this report is likely underestimated. The diagnosis was based on selective screening. Therefore, asymptomatic and pauci-symptomatic patients, those with severe phenotype, and early death were missed in the screening. Incidence reported from systematic screening is more accurate than those from selective screening. For example, in Japan, the incidence of severe PA by selective screening was 1:465,000, but was noticeably higher (1:17,400) when pauci-symptomatic patients were identified through systematic newborn screening (NBS).[40] The incidence of MMA was reduced from 44% with selective screening to 18.4% with NBS.[7] On the contrary, in Germany, both MMA and PA detected through selective screening considerably decreased when identified through NBS.[41,42] Therefore, it is difficult to completely compare the results of selective screening with those of NBS. In the current study, the detection of several OADs would have been missed due to (1) early death of severely affected patients, (2) asymptomatic or pauci-symptomatic forms, (3) low knowledge and awareness of physicians of these rare and non-specific phenotypically heterogeneous diseases, and (4) low availability and accessibility to screening and confirmatory tests.

In Tunisia, a single center offers biochemical diagnosis, and no center performs genetic testing for OADs in routine practice. The estimated incidence can be considerably influenced by the population targeted by the screening. For example, half of the patients diagnosed with 2-HGA over the 35 years were detected in 3 years (2009-2011) at the occasion of a program of screening in some centers for mental retardation. Furthermore, the disease does not present an acute deterioration course, which leads to a delayed clinical diagnosis in childhood, as most patients can live to adulthood.[43] The incidence also varies according to the detection capability of each center, which is influenced by the expertise of the medical team, technology, and methods available for the screening and confirmatory tests, accessibility of patients to testing, as well as financial resources of the center and the country.[44] In the current study, the screening was selective and was based on familial history or clinical presentation.

Table 2: Profile of organic aciduria diseases reported in different populations based on selective screening or systematic newborn screening

Author, year (reference)	Country	Type	Period	*n*	Organic acidurias profile											
					MMA	PA	2-HGA	GA-I	PGA	IVA	BKT	CD	AKU	3-MCC	MCD	Other
Haworth *et al*., 1991[35]	Canada	SS	1960–1990	29	ND	ND	3.44	48.27	ND	ND	ND	ND	ND	ND	3.44	44.82
Wajner *et al*., 2009[34]	Brazil	SS	1994–2008	161	21.11	11.18	5.59	20.49	ND	4.34	0.62	2.48	3.10	1.24	5.59	24.26
Echeverri *et al*., 2018[20]	Colombia	SS	2007–2017	61	13.1	22.9		26.2		9.84			3.28		4.92	9.84
Lehnert, 1994[41]	Germany	SS	1973–1994	151	22.51	21.85	0.66	4.63	3.97	10.59	5.29	2.64	3.31	0.66	ND	27.86
Saudubray *et al*., 1989[45]	France	SS	1968–1987	88	35.22	23.86	ND	ND	12.5	15.90	ND	ND	ND	ND	12.50	12.50
Shibata *et al*., 2018[7]	Japan	SS	2000–2015	184	44.02	13.04	2.17	9.23	0.54	1.08	1.08	ND	2.71	4.34	13.04	13.63
Yang *et al*., 2008[46]	China	SS	1998–2007	162	63.7	8.80		3.8		1.6	1.6		0.5	1.1	8.2	8.7
Sun *et al*., 2011[15]	China	SS	2006–2011	36	58.3	11.11	ND	5.55	ND	2.77	ND	ND	ND	2.77	ND	19.5
Sherazi *et al*., 2017[47]	Pakistan	SS	2013–2014	41	21.95	9.75	ND	7.31	ND	7.31	ND	4.87	4.87	ND	7.31	36.63
Keyfi *et al*., 2018[38]	Iran	SS	2006–2016	279	36.56	2.86	0.35	11.46	0.71	27.95	1.43	0.35	1.07	ND	ND	17.95
Al Riyami *et al*., 2012[21]	Oman	SS	1998–2008	53	9.43	26.41	ND	1.88	1.88	18.86	3.77	ND	ND	ND	ND	39.65
Golbahar *et al*., 2013[22]	Bahrain	SS	2008–2011	12	25	33.33	ND	16.66	ND	ND	ND	ND	ND	ND	ND	25
Karam *et al*., 2013[9]	Lebanon	SS	1998–2010	83	40.96	9.6	ND	2.4	ND	13.30	3.6	6.0	2.4	3.6	ND	18.14
Selim *et al*., 2014[10]	Egypt	SS	2008–2013	70	32.85	15.7	ND	8.57	ND	11.42	8.57	ND	ND	5.71	ND	17.18
Current study	Tunisia	SS	1987–2022	471	31.00	19.1	11.5	8.5	6.4	5.7	3.6	3.4	3.0	2.1	0.6	2.8
Frazier *et al*., 2006[48]	USA	NBS	1997–2005	58	17.24	5.17	ND	8.62	ND	12.06	3.44	ND	ND	44.8	ND	8.6
Lindner *et al*., 2011[42]	Germany	NBS	1999–2009	37	10.81	10.81	ND	16.21	ND	40.54	ND	ND	ND	21.62	ND	0
La Marca *et al*., 2008[49]	Italy	NBS	2002–2008	9	22.22	22.22	ND	11.11	ND	11.11	ND	ND	ND	33.33	ND	ND
Vilarinho *et al*., 2010[50]	Portugal	NBS	2004–2008	24	8.33	4.16	ND	25	ND	12.5	ND	ND	ND	29.16	ND	20.83
Shibata *et al*., 2018[7]	Japan	NBS	1997–2015	152	18.4	53.9	-	3.1	-	3.3	0	-	-	14.5	2.0	-
Al-Jasmi *et al*., 2016[51]	UAE	NBS	2011–2014	41	14.63	7.31	ND	7.31	ND	2.43	2.43	ND	ND	43.9	ND	21.99
Moammar *et al*., 2010[24]	KSA	NBS	1983–2008	43	32.55	13.95	13.95	6.97	ND	13.95	ND	6.97	ND	6.97	4.65	0

2-HGA – 2-hydroxyglutatric aciduria; 3MCC – 3-methylcrotonyl-CoA carboxylase deficiency; AKU – Alkaptonuria; BKT – β-ketothiolase deficiency; CD – Canavan disease; GA-I – Glutaric aciduria type 1; IVA – Isovaleric aciduria; KSA – Kingdom of Saudi Arabia; MMA – Methylmalonic aciduria; MCD – Multiple carboxylase deficiency; n – Number of reported cases; NBS – Newborn systematic screening; PA – Propionic aciduria; PGA – Pyro glutamic aciduria; SS – Selective screening; UAE – United Arab Emirates; USA – United States of America; ND – Not determined

In our series, the diagnosis was generally too late (median: 36 months). Patients' age at diagnosis varied from 1 day to 49 years. This large variation is partly linked to the varying age of expression of the type and the form of the disorder. Some OADs manifest in the first days of life, while others may manifest at a later age. More than 50% of patients with PA and PGA, 37% with IVA, and 30% with MMA were diagnosed within the first month of life, while all patients with Canavan disease and alkaptonuria were diagnosed during late childhood or adulthood. Late diagnosis is mainly due to limited knowledge/awareness of the physicians of these rare disorders, and low accessibility to biochemical and genetic diagnosis. The late diagnosis would seriously limit the chances for successful treatment and would increase the probability of the development of irreversible damage, particularly in the brain, which, in most cases, can change the natural course of the disease (e.g. 2-HGA, GAI, PA, and IVA).

The clinical and biochemical features in Tunisian OAD patients are like those reported in other populations. However, hypotonia was particularly frequent in our patients, likely due to a late diagnosis that caused irreversible neurological deterioration.[44] Metabolic acidosis and ketonuria were the most frequent biochemical abnormalities observed in our patients.

Patients' management, immediate outcome, compliance to treatment, and neurodevelopmental outcomes were rarely documented. Clinical data were derived from a pre-established form received with the samples. Analysis requests came from public and private facilities all over the country. Therefore, it is difficult to maintain contact with clinicians to track the clinical histories and outcomes. When documented, the management of patients was variable according to the resources of the health facility and the socioeconomic status of the family. Few patients benefited from adequate care with good compliance to diet and specific preparations and accurate biochemical monitoring. The management was inadequate for most patients due to difficulties in adhering to a low-protein diet and obtaining special preparations.

Strengths and limitations

The patterns and specific incidences of OAD reported herein would be close to the real incidence, as almost all Tunisian patients with OAD were included in this study. Indeed, Rabta Hospital is the only referral center in Tunisia that offers biochemical diagnoses for OAD. However, both the pattern and incidence of OADs are likely affected by the selective mode of screening, the low expertise of clinicians in the field, and the low accessibility to biochemical and genetic testing. The lack of data on patient management and outcomes is another limitation of the report.

CONCLUSIONS

This study found that MMA and PA together accounted for 50% of all OADs in Tunisia. In addition, the incidences of 2-HGA and PGA are among the highest in the world. The study also revealed that in general, the diagnosis was made very late, and that there was poor management of patients with OAD due to difficulties in following diet restrictions and obtaining costly special preparations.

Recommendations

Late diagnosis and poor management of OADs drastically limit the likeliness for clinical improvement and increase the risk of neurological deterioration in patients, and thus efforts should be undertaken to improve diagnosis capacities and management of OADs. Characterization of causative mutations will make prenatal diagnosis possible in affected families. The findings of this study also warrant the implementation of NBS for the diagnosis of OADs and other frequent and treatable neonatal disorders, which would allow determining the real incidence of OADs and enhance the likeliness of better prognosis. Pending the implementation of NBS in Tunisia, it is recommended to make efforts for early diagnoses of OADs in severely ill children because of the high prevalence of such disorders in these high-risk groups, and particularly, because of the availability of therapy for many of these disorders.

Ethical considerations

The protocol of the study was approved by the Ethics Committee of Rabta Hospital (Ref. no.: 20/HRT/14; date: April 17, 2020). Requirement for patient consent was waived owing to the study design. The study adhered to the principles of the Declaration of Helsinki, 2013.

Peer review

This article was peer-reviewed by two independent and anonymous reviewers.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

Conceptualization: A.J., H.S., and M.F.; Methodology: F.N., E.T., M.B.H., and R.G.; Data analysis: A.J., E.T., and M.F.; Writing–original draft preparation: A.J.; Writing – review and editing: M.F., H.S., and N.K.; Supervision: N.K. and S.H.T.

All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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