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SECRETARIA DE SALUD
INSTITUTO NACIONAL DE PEDIATRÍA

INBORN ERRORS OF INTERMEDIARY METABOLISM IN CRITICALLY Ill NEWBORNS IN MEXICO

T E S I S

PARA OBTENER EL TITULO DE:

MEDICO ESPECIALISTA EN PEDIATRIA

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MÉXICO, D.F. MAYO , 2014.

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INBORN ERRORS OF INTERMEDIARY METABOLISM IN CRITICALLY III

NEWBORNS IN MEXICO

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ESTE TRABAJO SE REALIZO EN EL LABORATORIO DE

ERRORES INNATOS DEL METABOLISMO Y TAMIZ DEL

INSTITUTO NACIONAL DE PEDIATRIA.

AGRADECIMIENTO:

Al apoyo técnico y asesoría en la elaboración de esta tesis a la

M. En C. Isabel Ibarra González de la Unidad de Genética de la Nutrición

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INBORN ERRORS OF INTERMEDIARY METABOLISM IN CRITICALLY ILL NEWBORNS FROM MEXICO

Abstract:

Inborn errors of intermediary metabolism (IEiM) are complex diseases with high clinical heterogeneity and some patients who have severe enzyme deficiencies or are subjected to stress (catabolism/infections) actually decompensate in the neonatal period. In this study we performed metabolic tests to 2025 newborns admitted to 35 intensive care units or emergency wards NICU/EW over a 6-year period in Mexico, in whom a metabolic disorder was clinically suspected; Of these 2025 sick newborns, 11 had an IEiM, revealing a prevalence of 1:184. Clinical characteristics and outcomes of the newborns with confirmed IEiM are shown. Of these 11 patients, 4 had isolated methylmalonic acidemia, 3 had maple syrup urine disease; 2 had urea cycle disorders; and 1 each had 3-hydroxy-3-methylglutaric acidemia and isovaleric acidemia. During the first week of life (average 3 days), all of these newborns presented with impaired alertness, hypotonia, feeding difficulties and vomiting, along with metabolic acidosis and hyperammonemia. Of the 11 newborns with an IEiM, 7 died, making the mortality rate 64%. In conclusion, the differential diagnosis of newborns admitted to the NICU/EW must include IEiM, requiring systematic screening of this population.

Key words: Inborn errors of metabolism; neonatal intensive care unit; neonatal mortality; newborn screening, neonates.

Introduction

Inborn errors of intermediary metabolism (IEiM) are a complex, heterogeneous group of genetic diseases. Most of them have severe neonatal onset and are a primary cause of death in newborns and infants. Unfortunately, newborns have a limited variety of responses to illness, and the early signs and symptoms of IEiM are similar to the features of other, more common neonatal illnesses.[1] Specific and effective treatments are available for many IEiM, and early therapeutic intervention can prevent the worsening of disease. Even if therapy is unavailable, an accurate diagnosis is crucial for genetic counseling.[2]

IEiM represent a challenge to physicians. Prompt suspicion of these diseases in sick newborns and knowledge of initial diagnostic approaches can aid in the selection of appropriate biochemical tests and measures of emergency management.[3] However, the very early clinical onset of the severe neonatal forms of some of these disorders represent a logistics challenge to newborn screening programs. Neonates with severe IEiM often become seriously ill during the first days of life and must be admitted to intensive care units or emergency wards (NICUs/EWs).[4,5,6]

Most children born in developing countries are not subject to mandatory neonatal screening for IEiM, making prompt and accurate diagnosis critical as soon as their clinical manifestations appear.[7] The Inborn Errors of Metabolism and Screening Laboratory (LEIMyT) of the National Institute of Pediatrics (INP) in Mexico City, is a national reference center devoted to the diagnosis and management of

aminoacidopathies, organic acidemias and fatty acid oxidation disorders.[8] The objective of the present study was to assess the prevalence of IEiM in newborns admitted to neonatal intensive care units and emergency wards (NICU/EW) throughout Mexico, whom a metabolic disorder was suspected and to describe their clinical characteristics and the outcomes of the first episode of metabolic decompensation.

Methods

Metabolic tests were performed to 2025 newborns (less than 30 days old at the time of the sample collection) in whom a metabolic disorder was clinically suspected, who were admitted to 35 intensive care units or emergency wards NICU/EW, 23 in the Mexico City metropolitan area and 12 in other Mexican States from January 2007 to December 2012. Suspicion of a metabolic disorder was based on the clinical findings in each patient; e.g. unexplained neurological signs and/or digestive symptoms and/or cardiac signs and/or positive family history; or unexplained biochemical abnormalities, such as metabolic acidosis, hyperammonemia or liver failure. Metabolic diagnostic tests included analysis of urinary organic acids, determination of plasma amino acid concentrations and the quantification of amino acid and acylcarnitine concentrations in dried blood spot samples by tandem mass spectrometry (MSMS) which was introduced in our laboratory since January 2010. The characteristic metabolic profile with the primary and secondary markers description, as well as the confirmatory tests was based on

previously published data.[9,10] The markers were defined according to Millington et al.,[11] as following: primary markers are those having potential diagnostic significance, while secondary ones are those biomarkers adding an element of risk for an underlying metabolic disorder, including certain biochemically related analyte ratios.

The frequency of IEiM was calculated as the ratio of newborns diagnosed to those suspected of having an IEiM. The study was approved by the INP Institutional Review Board, approval number 017/2011.

Urinary organic acid analysis.

The concentrations of organic acids in urine were determined by gas chromatography–mass spectrometry (GC/MS) on an Agilent™ 6890N gas chromatograph coupled to an MSD 5973 mass spectrometer as described by Sweetman L.[12]

Quantification of plasma amino acids.

Amino acid analysis was performed using reverse phase high performance liquid chromatography (HPLC), with fluorescence detection using an automated Waters™ system.[13]

Quantification of amino acids, acylcarnitines and succinylacetone.

Dried blood spots were analyzed by electrospray ionization liquid chromatography-tandem mass spectrometry (LC-MS/MS), using a Quattro Micro API (MicroMass™) tandem mass spectrometer with commercial kits (NeoBase™ Non-derivatized MSMS Kit, PerkinElmer™)

Results

During the studied period, a total of 2025 samples from newborns admitted to NICU/EW in whom an IEiM was suspected were analyzed; the proportion of premature vs. full term newborns was 33% vs 77% respectively; from these samples, eleven patients 8 male and 3 female, were diagnosed with 6 different IEiM. Four newborns were diagnosed with isolated methylmalonic acidemia (iMMA); three with maple syrup urine disease (MSUD); two with urea cycle disorders (UCD), including one each with ornithine transcarbamylase deficiency (OTCD) and argininosuccinic acidemia (ASA); and one each with 3-hydroxy-3-methylglutaric acidemia (HMG) and isovaleric acidemia (IVA). Non fatty acid oxidation disorder (FAO) was found in the studied patients. The prevalence of IEiMs in sick newborns was 1:184; Amino acid, acylcarnitine and ratios determined by MSMS as well as the confirmatory test findings are shown in Table 1. Consanguinity was found in 2 families (18%). All the IEiM confirmed had an autosomal recessive inheritance, except 1 OTC case (X-linked inheritance). 4 patients (36%) had a previously affected sibling who died with similar clinical manifestations, with one diagnosed postmortem with IVA. All 11 newborns were full-term and born after normal pregnancy and delivery. Ten were discharged after birth as healthy newborns, whereas one patient with OTCD was kept in the hospital due to intrauterine growth delay and cephalohematoma.

The average age at the onset of symptoms was 3 days, and the mean gap between onset of symptoms and diagnosis was 14.5 days. Six of the 11 patients

died during the first hospitalization; whereas one was diagnosed postmortem (Table 2).

The main clinical features of this group of newborns are shown in Table 3. Neurological symptoms were the most frequent event in all patients, followed by abnormalities in biochemical blood markers. Typical clinical manifestations included impaired alertness, stupor or coma, hypotonia, unexplained feeding difficulties and vomiting accompanied with metabolic acidosis and hyperammonemia, all occurring during the first week of life. Table 4 compares the IEiM detection and consanguinity rates in our population with similar studies performed on other populations.

Discussion

Although several studies have assessed the frequency of IEiM in sick children,[14-17] only few others have analyzed its occurrence specifically in critically ill newborns.[4,18] Our study population included critically ill newborns admitted to NICU/EW and suspected of having a genetic metabolic disorder upon hospitalization. The prevalence of IEiM in this group was 1 in 184 newborns, less than the rates in Bahrain (1:79) and Oman (1:4); However, the consanguinity of those populations (84% and 81%, respectively)[14,18] was more frequent than in ours (18%).

iMMA was the most frequently detected genetic disorder in these patients, being present in 1 in 506 sick newborns. It is known that iMMA is one of the most

common IEiM detected worldwide, both in high risk patients,[8,16,18-20] and during screening of asymptomatic newborns.[21]

It is noteworthy that not a single FAO was detected in the population studied, this could be due to some reasons: a) it is known that some patients among this group of disorders can present sudden death syndrome and they die without diagnosis;[22] b) false negative result due to carnitine depletion of the patient or because the sample was not collected at the moment of metabolic decompensation;[23] c) low prevalence of these disorders among Hispanic population as has been documented in some newborn screening programs.[21]

Symptom onset was observed very early, at an average of 3 days of life (range 0.5-6 days); All patients with an IEiM manifested the disease during the first week of life after an initial symptom free period even one showing signs as early as 12 hours of life. The median gap between the onset of symptoms and a definitive diagnosis was 14.5 days (7-28.5 days), interestingly; the patient whose symptoms began earliest had the longest delay in diagnosis (28.5 days). This patient initially presented with irritability, dehydration and fever, later developing abdominal distention, spasticity, seizures and apnea requiring mechanical ventilation. He was initially thought to have neonatal sepsis but due to adverse outcome he was suspected at age 20 days of having an IEiM. A diagnosis of MSUD was confirmed at age 29 days and he died at age 2.3 months.

Table 1. Metabolic screening and confirmatory tests resçults of the studied cases.

Diagnosis	Confirmed cases	Abnormal analytes in MSMS					Confirmatory test findings
Methylmalonic acidemia (iMMA)	RV	↑C3 5μM		↑C3/C2 (R) 0.26		↑C3/C0 (R) 0.2	Organic acid urine profile: ↑Methylmalonate,↑methylcitrate, ↑3-hydroxypropionate, ↑propionylglycine
	1	7.0		1.0		3.9	
	1	7.7		1.1		4.9	
	1	13.9		1.7		10.8	
	1	ND		ND		ND	
Maple syrup urine disease (MSUD)	RV	Xle 250μM	Val 219μM	Xle/PHE (R) 4.61	Xle/Ala (R) 1.19	Val/Phe (R) 3.25	Amino acid plasma profile: ↑Leu, ↑Ile, ↑Val. Organic acid urine profile: ↑2ketoisocaproate, ↑2-ketoisovalerate, ↑2-keto-3-methylvalerate, ↑2hydroxyisocaproate, ↑2hydroxyisovalerate, ↑2-hydroxy-3methylvalerate, ↑phenyllactate, ↑phenylpyruvate
	1	673	61	16.22	6.04	1.46	
	1	3199	277	91.63	45.3	8.03	
	1	2437	302	62.34	26.54	6.93	
Isovaleric acidemia (IVA)	RV	C5 0.5μM		C5/C0 (R) 0.02	C5/C2 (R) 0.04	C5/C3 (R) 0.39	Organic acid urine profile: ↑3-Hydroxyisovalerate ↑isovalerylglycine
	1	6.45		0.6	1.79	25.25	
Ornithine transcarbamylase deficiency (OTCD)	1	Sample not analyzed by MSMS					Amino acid plasma profile: ↓Citruline ↑Urinary orotate
3-Hydroxy-3-methylglutaric aciduria (HMG)	1	Sample not analyzed by MSMS					Organic acid urine profile: ↑3-Hydroxy-3-methylglutarate, ↑3-methylglutaconate, ↑3-methylglutarate, ↑3-hydroxyisovalerate, ↑3-methylcrotonylglycine
Argininosuccinic acidemia (ASA)	1	Sample not analyzed by MSMS					Amino acid plasma profile: ↑Argininosuccinic acid, ↑Citruline

Abbreviations. R: ratio; RV: Reference value; C3: Propionylcarnitine; C2: Acetylcarnitine; C0: Free carnitine; Xle: Leucine+isoleucine+alloisoleucine+norleucine+hydroxyproline; Val: Valine; Phe: Phenylalanine; Ala: Alanine; C5: Isovaleryl/2-methylbutyrylcarnitine.

Table 2. Age at clinical onset, diagnosis, gap and status at discharge from the neonatal intensive care unit and emergency wards (NICU/EW) of 11 Mexican newborns with IEiM.

Patient number	Diagnosis	Sex	A: age at clinical onset (days)	B: age at diagnosis (days)	B-A gap (days)	Status at NICU/EW discharge
1	Maple syrup urine disease	M	0.5	29	28.5	Dead
2	Argininosuccinic acidemia	M	1	14	13	Dead
3	iMethylmalonic acidemia	F	2	20	18	Alive
4	iMethylmalonic acidemia	F	2	10	8	Dead
5	3-Hydroxy-3-methylglutaric acidemia	M	2	16	14	Alive
6	Ornithine transcarbamylase deficiency	M	3	17	14	Dead
7	iMethylmalonic acidemia	F	3	20	17	Dead
8	Isovaleric acidemia	M	3	13	10	Alive
9	iMethylmalonic acidemia	M	5	18*	13	Dead
10	Maple syrup urine disease	M	5	22	17	Alive
11	Maple syrup urine disease	M	6	13	7	Dead
	Average		**3**	**17.4**	**14.5**	

* Postmortem diagnosis, death at 17 days of life.

Of the 11 patients with an IEiM, 7 (64%) died during their first hospitalization. The early onset of symptoms and high mortality rates in newborns with metabolic disorders, including organic acidemias and urea cycle disorders, have been documented in other populations of sick newborns, ranging from 29% in France[4] to 36% in Thailand[24] and 50% in China.[6] Moreover, many infants with IEiM die during their first episode of metabolic decompensation before diagnosis.[25] Fortunately, in some medical centers, the mortality rate during the first IEiM episode has decreased, due to rapid diagnosis and aggressive and timely treatment.[26,27]

It is interesting to note that within the patients found with autosomal recessive inheritance IEiM (10/11) there was a predominance of males (8/10). This has been reported in previous publications; Wasant et al., studied 35 patients, and found a male predominance of 2:1 and only 3 patients had an X-linked IEiM;[28] similarly, Marin-Valencia et al., found a male predominance of 1.4:1 among 42 studied newborns (3 patients with X-linked inheritance).[29] However, there are no further explanation that allows making any inference and the number of patients studied to date is too small for any generalization.

Table 3. Clinical characteristics of neonates diagnosed with an IEiM at neonatal intensive care units and emergency wards in Mexico.

	TOTAL	iMMA	MSUD	OTCD	ASA	HMG	IVA
Number of patients	11	4	3	1	1	1	1
Symptomatology							
Neurological							
• Impaired alertness, stupor or coma	11	4	3	1	1	1	1
• Abnormal tone of limbs or trunk	10	4	3		1	1	1
• Irritability	6	1	3	1			1
• Seizures	6	1	3	1			1
Biochemical abnormalities							
• Metabolic acidosis	8	4	2			1	1
• Hyperammonemia	8	3	2	1	1		1
• Ketosis	7	4	2				1
• Hypoglycemia	6	3	1			1	1
• Hyperlactatemia	4	3	1				
Gastrointestinal							
• Unexplained feeding difficulties and vomiting	9	4	3		1		1
• Hepatomegaly	1	1					
• Diarrhea	1		1				
Hematological (cytopenia, abnormal bleeding)	6	3	1		1		1
Impairment respiration/circulation	6	1	2	1		1	1
Other characteristics							
• Abnormal urine odor	4		3				1
• Dehydration	2		1			1	
Suggestive family history							
• Siblings with unexplained death	4	3					1
• Siblings with an IEiM previously diagnosed	1						1
• Consanguinity	2		1				1

Abbreviations: iMMA: isolated methylmalonic acidemia; MSUD: maple syrup urine disease; OTCD: ornithine transcarbamylase deficiency; ASA: argininosuccinic acidemia; HMG: 3-hydroxy-3-methyglutaric acidemia; IVA: isovaleric acidemia

Table 4. Comparison of IEiM detection (total and by individual disease) and consanguinity rate in 3 different populations of sick newborns.

IEiM	Detection rate		
	Present study Mexico n=2,025 NB	**Kingdom of Bahrain[18] n=1,986 NB**	**Oman[14] n=166 NB**
iMethylmalonic acidemia	1:506	1:662	1:166
Maple syrup urine disease	1:675	1:497	1:17
Urea cycle disorders	1:1012	1:1986	1:24
3-Hydroxy-3-methylglutaric acidemia	1:2025	NF	1:166
Isovaleric acidemia	1:2025	1:993	1:33
Total	**1:184**	**1: 79**	**1:4**
Consanguinity rate	18%	84%	81%

Abbreviations: NB: newborns; NF: not found in this series.

As expected, the clinical manifestations of IEiM were non-specific and generally multisystemic, affecting mainly the neurological, gastrointestinal, and hematological systems with impairments in respiration or circulation accompanied by biochemical abnormalities. These IEiM can result in acute or progressive intoxication, due to the accumulation of toxic compounds proximal to the metabolic block. However, these may manifest as non-specific symptoms, attributable to other common causes, including sepsis, cardiac failure or intraventricular hemorrhage.[1,30]

We also found that 4 of the 11 patients had suggestive family histories, including siblings who died unexpectedly, siblings previously diagnosed with an IEiM, or consanguineous parents (Table 3). The early onset, non-specificity of symptoms and high mortality rate of IEiM indicate that these diseases must be suspected and

considered during the differential diagnosis of any ill newborn admitted to the NICU or emergency ward, especially those with suggestive family history. High risk metabolic screening must be part of the initial medical approach for these patients.

Newborn screening has changed the natural course of some IEiM, preventing sequelae and diminishing mortality rates.[31] However, the severe neonatal forms of these disorders pose a challenge to newborn screening programs, as their potentially devastating clinical symptoms may be present during the first few days of life, even before the Guthrie card is collected or abnormal results are reported. Many screening programs require specimens to be collected after 24 hours of age, with results generally reported when infants are between 6 and 15 days of age, a time at which most of the infants with severe organic acidemias have become seriously ill.[32] Fortunately, highly sensitive and accurate techniques like MS/MS have shortened the turnaround time of diagnosis, allowing early collection of blood samples while providing immediate determination of amino acids, acylcarnitines and their ratios, resulting in earlier diagnosis and the prompt initiation of treatment.[33]

It is important to emphasize that any child with an abnormal neonatal screening result suggestive of an IEiM must be carefully evaluated and hospitalization must be considered, even in the absence of symptoms.[22] Rapid diagnostic confirmation and implementation of disease-specific treatment is essential for effective prevention.[33,34]

The IEIMs prevalence presented in this study is useful for appropriate newborn screening expansion of in Mexico.

Conclusion

All newborns admitted to NICU/EW must be immediately and systematically screened for IEiM. Emergency treatment protocols should be initiated as soon as a metabolic disorder is suspected, to reduce mortality rates and sequelae associated with these diseases.

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