Selective screening of 18,000 high-risk Brazilian patients for the detection of inborn errors of metabolism

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OBJECTIVE: The number of diagnosed inborn errors of metabolism (IEM) is growing constantly due to the improvement and widespread availability of analytical techniques. In 1982, a laboratory for the detection of IEM was set up in Porto Alegre, Brazil, and became a national reference centre for the diagnosis of these disorders. The aim of this study is to report the most frequent IEM diagnosed in our country.

MATERIAL AND METHODS: Eighteen thousand patients with signs and symptoms suggestive of IEM were investigated in our laboratory from 1982 to 2000 using specific protocols which included tests for the detection of glucosaminoglycans (GAGS), amino acids, sugars, oligosaccharides, sialyloligosaccharides, organic acids, as well as various metabolites.

RESULTS: The biochemical investigation was completed in 17,822 patients and an IEM was detected in 1,460 cases (8.5%). Groups of IEM of higher incidence in our sample were lysosomal storage disorders (59.4%) and aminoacidopathies (18.8%). The disorders most frequently diagnosed were Gaucher disease, GM1 gangliosidosis, mucopolysaccharidosis type I, classical phenylketonuria, mucopolysaccharidosis type VI and mucopolysaccharidosis type II.

CONCLUSIONS: This study shows that the establishment of reference centres for the investigation of rare genetic diseases is a suitable approach to the study of IEM in developing countries such as Brazil.

Key-words: Inborn errors of metabolism; selective screening; biochemical genetics.

Investigação seletiva de 18 mil pacientes brasileiros de alto risco para a detecção de erros inatos do metabolismo

OBJETIVOS: O número de erros inatos do metabolismo (EIM) diagnosticados está crescendo constantemente devido ao aperfeiçoamento e disponibilidade das técnicas laboratoriais. Em 1982, foi estabelecido em Porto Alegre, Brasil, um laboratório para a detecção de EIM e este tornou-se um centro de referência nacional para o diagnóstico destes distúrbios. O objetivo deste trabalho é registrar os EIM mais freqüentes diagnosticados em nosso país.

MATERIAL E MÉTODOS: Dezoito mil pacientes apresentando sinais e/ou sintomas sugestivos de um EIM foram investigados em nosso laboratório de 1982 a 2000, utilizando-se protocolos específicos que incluíam testes para a detecção de glicosaminoglicanos (GAGS), aminoácidos, açúcares, oligossacarídios, sialiloligossacarídios, ácidos orgânicos e outros metabólitos.

RESULTADO: Dezessete mil oitocentos e vinte e dois pacientes completaram a imvestigação bioquímica e em 1.460 casos (8,5%) foi detectado um EIM. Os grupos de EIM de maior

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freqüência em nossa amostra foram as doenças lisossômicas de depósito (59,4%) e as aminoacidopatias (18,8%). Os distúrbios mais freqüentes foram a doença de Gaucher, a gangliosidose GM1, a mucopolissacaridose tipo I, a fenilcetonúria clássica, a mucopolissacaridose tipo VI e a mucopolissacaridose tipo II.
CONCLUSÕES: Esse estudo mostrou que o estabelecimento de centros de referência para a investigação de doenças genéticas raras é adequado para o estudo de EIM em países em desenvolvimento como o Brasil.

Unitermos: Erros inatos do metabolismo; investigação seletiva; genética bioquímica.

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Introduction

Specific protocols for selective screening of inborn errors of metabolism (IEM) in high-risk patients have been introduced since 1950 in several countries. In the initial surveys, the techniques employed were simple and could only detect a few diseases. A constant improvement of analytical equipment and techniques for assaying metabolites has allowed the diagnosis of an increasing number of disorders. A further improvement in the detection of these diseases has been achieved by the introduction of more elaborate procedures such as tissue culture, specific enzyme assays and molecular analyses (1).

The establishment of an accurate diagnosis is necessary for the introduction of supportive and/or specific therapeutic measures. When a successful treatment is not available, genetic counseling and prenatal diagnosis can be offered in order to prevent new cases in the index family. Therefore, selective screening for inherited metabolic diseases represents a major challenge to modern preventive medicine (1). Reference centres for selective screening were set up in developed countries and, in most cases, became specialized in the detection of specific groups (such as aminoacidopathies, organic acidaemias, lysosomal storage disorders (LSD), peroxisomal disorders, etc.) or even for the diagnoses of a single disease.
Based on the experience of developed countries, a reference laboratory for the detection and diagnosis of IEM was set up in Porto Alegre, Southern Brazil. The laboratory is part of a University Hospital and has been receiving biological samples from patients at risk for IEM from all over Brazil since 1982.

We present here the results obtained from analysis of samples from 18,000 high-risk patients referred to our centre from January 1982 to December 2000. In addition, we discuss the role of a reference laboratory for IEM in developing countries.

Material and methods

The patients studied were referred for investigation by several services from different regions of Brazil and from other countries in Latin America.

Urine and plasma samples were obtained from 18,000 patients with signs and/or symptoms suggestive of a metabolic disorder and at first they were submitted to the “basic” protocol. The tests included in this protocol (table 1) comprise qualitative screening tests of urine and amino acid paper chromatography of plasma and urine (2,3). Further investigations were carried out in the case of a positive or doubtful result in any of these tests, or when the specific metabolic disorder suspected was not investigated by tests included in this “basic” protocol. In these cases, additional biological samples were requested, such as white blood cells (WBC), cerebrospinal fluid (CSF) and/or skin biopsy, and analyzed by selected specific assays of the “extended” protocol (table 2). In addition, specific enzyme assays on leukocytes, erythrocytes, skin fibroblasts or liver biopsy were performed for confirmation of a diagnosis (table 3). Occasionally, samples were sent to reference laboratories in other countries if complementary analyses became essential.

Results

Table 4 summarizes the diagnoses performed in the current study. The results were

Table 1. Qualitative tests included in the "basic" protocol

Qualitative tests	Sample	Metabolites Detected
Benedict	Urine	Reducing substances
Ferric chioride	Urine	Aromatic hydroxyl groups
Nitrosonaphthol	Urine	Tyrosine and its metabolites
p-Nitroaniline	Urine	Methvlmalonic and ethylmalonic acids
Cyanide-nitroprusside	Urine	Cystine and homocystine
CTMA bromide	Urine	Glycosaminoglycans
Dinitrophenylhydrazine	Urine	Keto acids
Paper chromatography	Urine/plasma	Amino acids
Sulfite test	Urine	Deficiency of molibidenium cofactor

Table 2. Tests included in the "extended" protocol

Test	Detection of	Sample
Toluidine blue spot test	Glycosaminoglicans	Urine
Thin-layer chromatography	Oligosaccharides	Urine
	Givcosaminoglycans	Urine
	Sialyloligosaccharides	Urine
	Carbohydrates	Urine
High performance liquid chromatography	Amino acids	Plasma, Urine, CSF
Gas chromatography	Organic acids	Urine
Quantitative assays	Orotic acid	Urine
	Sialic acid	Urine
	Glycogen	WBC
	Succinylacetone	Plasma
	Thiosulphate	Urine
Filipin staining test for Niemann-Pick C disease	Cholesterol Storage	Skin fibroblasts

obtained by the evaluation of 17,822 patients who completed the investigation. The study confirmed the presence of 80 different metabolic disorders in 1,460 patients, corresponding to a frequency of 8.19%. These diagnoses were distributed among the various groups of disorders, more than half of them being represented by the LSD group (59.4%). Other groups included aminoacidopathies (18.8%), organic acidaemias (6.9%) and disorders of carbohydrate metabolism (5.3%). Among the disorders most frequently diagnosed were Gaucher disease (13.5%), GM1 gangliosidosis (7.05%), mucopolysaccharidosis type I (5.5%), classical phenylketonuria (PKU) (5.5%), mucopolysaccharidosis tvpe VI (4.4%) and mucopolysaccharidosis type II (4.3%).

Table 3. Specific enzymatic assays performed in this study in selected cases

Enzyme	Sample	Disease
α-Fucosidase	L, F	Fucosidosis
α-Galactosidase	P	Fabry disease
α-Glucosidase	L, F	Pompe disease
α-Iduronidase	P	Mucopolysaccharidosis I
α-Mannosidase	L, F	α-Mannosidosis
Acetyl-CoA glucosaminide N		
-acetyltransferase	L, F	Mucopolysaccharidosis IIIC
Arylsulphatase A	L, F	Metachromatic leukodystrophy, MSD
Arylsulphatase B	L, F	Mucopolysaccharidosis VI, MSD
Arylsulphatase C	F	X-linked ichthyosis, MSD
β-Galactosidase	L, F	GM1 gangliosidosis, Mucopolysaccharidosis IVB
β-Glucosidase	L, F	Gaucher disease
β-Glucuronidase	P, L	Mucopolysaccharidosis VII, Mucolipidosis
β-Mannosidose	L, F	β-Mannosidosis
Biotinidase	P	Biotinidase deficiency
Ceruloplasmin	P	Wilson disease, Menkes disease
Fructose-1,6-diphosphatase	LB	Fructose-1,6-diphosphatase deficiency
Galactosylceramidase	L, F	Krabbe disease
Galactose-1-P-uridyltransferase	E	Classical Galactosaemia
Galactose-6-sulphatase	L, F	Mucopolysaccharidosis IVA, MSD
Glucose-6-phosphatase	LB	Glycogenosis type I
Heparan sulphamidase	F	Mucopolysaccharidosis IIIA
Hexosaminidases	P, L, F	Tay-Sachs disease, Sandhoff disease, Mucolipidosis
Iduronate sulphatase	P	Mucopolysaccharidosis II, MSD
N-acetylgalactosaminidase	P	Schindler disease
N-acetylglucosam-6-sulphatase	L, F	Mucopolysaccharidosis IIID
N-acetylglucosaminidase	P	Mucopolysaccharidosis IIIB
Neuraminidase	F	Sialidosis
Sphimgomyelinase	L, F	Niemann-Pick disease types A and B

(*P* plasma, *L* leucocytes, *F* skin fibroblasts, *E* erythrocytes, *LB* liver biopsy, *MSD* multiple sulphataase deficiency)

Discussion

The estimated frequency of IEM in high-risk patients established in this study was 8.19%. This value is higher than the value established in a previous study of our group developed in 1997 with 10.000 patients (4). Another study reported by Wannmacher et al. (5), which analyzed a population from the same region of Brazil, estimated this frequency as being 5.9%. In addition, a study carried out by Chamoles et al. (6) in Argentina found a frequency of IEM of 6.25% among 14,928 high-risk patients carrying a metabolic abnormality. We attribute the difference among these frequencies to high specialization of our laboratory in these last years. At this time, we are capable of diagnosing a large group of metabolic diseases and we have been in contact with several centres in Europe and USA that help us when necessary.

In a previous study by our group (4,7), GM1 gangliosidosis was the disease with the highest incidence in our population. In the present investigation, Gaucher disease and GM1 gangliosidosis are the most frequent IEM diagnosed. The increase in the diagnosis of Gaucher disease is due to a specific program of detection of this disease developed by our group in the last two years, since this disease has an efficient treatment with enzymatic reposition therapy. GM1 gangliosidosis also has a high prevalence in our region what is in accordance with the data of Severini et al. (8).

The mucopolysaccharidosis (MPS) type I and VI are the most frequent MPS found in the present study and MPS II appear in third position. The increase in the MPS II frequency is due to the improvement of its laboratory diagnosis, i. e, the analysis of the iduronate sulphatase activity, the enzyme deficient in this condition.

PKU has also been increasingly diagnosed in our laboratory, probably as the result of an increase in the proportion of newborns being referred to us because of the mass neonatal screening for PKU in our country. The same fact has been also observed in other Latin American countries like Chile and Mexico (9,10).

LSD were detected in 59.4% of our sample, representing the most frequent IEM group. Similar results have also been observed in Colombia, where this group of diseases, mainly represented by mucopolysaccharidosis, is more frequently reported (11). On the other hand, in a different survey conducted by Velázquez et al. (12), using the questionnaire of the Metabolic Information Network (MIN), LSD and amino acid disorders were found to be the most frequent disorders in Latin America. The questionnaires used in this survey were completed by physicians from different Latin America countries (Argentina, Brazil, Chile, Colombia, Costa Rica, Cuba, Mexico and Venezuela).

We should emphasize that our laboratory was the first to offer specific diagnosis of LSD in Brazil and soon became recognized as a specialized centre for these disorders, and this has probably contributed to the high frequency of the diseases found in the present study. Moreover, storage disorders are more “evident” to the clinician since they usually cause “syndromic” (coarse) facies, hepato and/or splenomegaly, macrocephaly or other signs that call the attention of clinicians.

Other studies on the prevalence of IEM in European populations revealed that aminoacidopathies and organic acidaemias are the most frequent disorders among the IEM (1). Since we have set up high-performance liquid chromatography and ion-exchange chromatography for aminoacid quantification and gas chromatography for organic acid detection, the number of aminoacidopathies and especially organic acid disorders identified has grown steadily.

Specifically regarding organic acidaemias, the rapid diagnosis of these clinically severe diseases in our laboratory has permitted prompt treatment in many cases and saved some lives. Before our facilities for organic acid detection were set up, suspected samples were sent abroad and not uncommonly many patients died before the diagnosis was made. This emphasizes the importance of the local establishment of techniques for the detection of severe and lethal disorders for which effective therapy is available.

The rarity, heterogeneity and complexity of IEM are general problems to be addressed by services that work on the diagnosis of these diseases. The investigation of high-risk patients in specialized centres, allowing the combination of relatively low investment and high technical quality, seems to be an adequate alternative for Brazil, which is potentially applicable to other developing countries.

Table 4. IEM diagnosed in the 17,822 patients who completed the investigation

Group	IEM	n	%
Lysosomal			
Storage disorders	Mucopolysaccharidosis		867
	I	81	
	II	63	
	VI	65	
	IIIA, B, C	57	
	IVA, B	52	
	VII	6	
	Not classified	47	
	Gaucher disease	197	
	GM1 gangliosidosis	103	
	Metachromatic Leukodystrophy	48	
	Niemann-Pick disease Type A, B or C	43	
	Krabbe disease	27	
	GM2 galgliosidosis		
	Tay-Sachs disease	25	
	Sandhoff disease	9	
	Mucolipidosis type II or III	14	
	Fabry disease	13	
	Galactosialisosis	9	
	Sialidosis	6	
	Multiple sulphatase Deficiency	2	
Amino acid disorders			274
	Hyperphenylalaninaemia Not classified	80	
	Classical PKU	66	
	Homocystinuria	36	
	Maple syrup urine disease	29	
	Non-ketotic hyperglycinaemia	15	
	Transient Hyperphenylalaninemia	11	
	BH4 metabolism defects	8	
	Hereditary tyrosinaemia	7	
	Others	22	101
Organic acidaemias			
	Lactic acidaemia	19	
	Methylmalonic acidaemia	16	
	3-OH-3ME glutaric acidury	11	
	Glutaric acidaemia	11	
	Primary hyperoxaluria	10	
	Propionic acidaemia	8	
	Others	26	
Carbohydrate disorders			77
	Gycogenosis	39	
	Galactosaemia	37	
	Pentosuria	1	
Miscellaneous			
a) Transport			
	Cystinuria	23	33
	Hypophosphataemic rickets	6	
	Others	4	
b)Peroxisomal			
	X-linked adrenoleukodystrophy	28	39
	Zellweger syndrome	4	
	Others	7	
c) Metal and heme, others	Porphyria	13	69
	X-linked ichthyosis	9	
	Lesch-Nyhan syndrome	5	
	Others	42	
Total		964	100

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