Inborn Errors of Metabolism, an Important Group of Orphan Neglected Diseases: Investigation of 8,000 Patients in Rio de Janeiro, Brazil

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Doenças raras negligenciadas, como erros inatos do metabolismo (IEM), afetam uma parcela pequena da população, mas necessitam de mais atenção da saúde pública no Brasil. Investigação de 8.000 pacientes em laboratório analítico de referência no Rio de Janeiro revelou 487 IEM (6,1%). O número de casos diagnosticados poderia ser maior se existissem mais laboratórios especializados.

Orphan neglected diseases, such as inborn errors of metabolism (IEM), affect a small percentage of the population. In Brazil, they need more attention from the public health system. Investigation of 8,000 patients in a reference analytical laboratory in Rio de Janeiro revealed 487 IEM (6.1%). The number of diagnosed cases could be larger if more specialized laboratories were available.

Keywords: inborn errors of metabolism, chemical analytical techniques, diagnosis, public policy

Introduction

Orphan neglected diseases are dysfunctions that affect a small percentage of the population. Many of them are of genetic nature. These rare diseases remain in an individual throughout their whole life and are often very serious or fatal. Developing medication for each of these disorders may represent a challenge due to its scarcity. Inborn errors of metabolism (IEM) belong to this category of diseases. There are more than 500 inherited metabolic diseases, individually rare, caused by genetic mutations. These mutations result in inactive or partially active proteins, which may alter synthesis, transport, degradation or storage of molecules in the organism.⁴

Inborn errors of metabolism may be classified into three great groups: group I, which leads to intoxication, group II, involving energetic metabolism, and group III, which comprises complex molecules. These diseases may develop in any phase of life, from prenatal to adult.⁵

Regarding group I, aminoacidopathies and organic acidemias are the most diagnosed in the first days and weeks of life in seriously ill children, making up over 100 disorders.⁶ In group II, mitochondrial diseases and beta-oxidation defects, among others, also make up over 100 disorders. Group III comprises peroxisomal diseases, glycosilation defects and lysosomal storage diseases (LSD), among others. LSD is the largest group, comprising 70 disorders.⁷

The interest for IEM has been growing exponentially worldwide, principally in the last decades. This is due mainly to the development of molecular biology and a new variety of modern analytical techniques. The new specialized Laboratory for Biochemical Diagnosis of IEM performs extended chemical analyses of biological fluid components (metabolic screening) of clinically selected patients. As such, hundreds of new dysfunctions could be recognized and characterized and new potential biomarkers are discovered continuously.⁸

Following initial physical investigation, simple chemical screening tests are carried out in urine to detect pertinent metabolites. Qualitative chromatographic techniques, including thin-layer chromatography (TLC) indicate the next analyses that should be performed. Samples with altered results are forwarded to more sensitive and specific
analytical techniques for identification and quantification of accumulated substances: ion exchange chromatography (automatic amino acid analyzer, AAA), high-performance liquid chromatography (HPLC), gas chromatography coupled to mass spectrometry (GC-MS) and tandem mass spectrometry (MS/MS). Enzymatic assays, to determine the activity of the deficient enzymes in various types of biological material, confirm diagnosis.

The purpose of this work is to present and disseminate the results of a survey of a number of diagnosed IEM cases in a reference laboratory in Rio de Janeiro, in the last 20 years, exposing it to society and the government, in a hope to stimulate public policies towards IEM.

**Experimental**

**Material**

Samples of urine and plasma were stored frozen at –20 °C until assayed. Most urine samples were occasional, and results were expressed as units of metabolites per g of creatinine.

**Reagents and equipments**

Reagents and solvents were purchased from Sigma-Aldrich (St. Louis, USA) and Tedia (São Paulo, Brazil); TLC plates from Merck (Darmstadt, Germany); filter paper from Whatman (Mainstone, England); HPLC (Agilent model 1100); GC-MS (GC: HP5890A Series II; MS: HP5792); Automatic AAA (Biochrom 20); UV-Vis spectrophotometer (Shimadzu UV-1601-PC).

**Methods**

Initially, samples were submitted to physical investigation (pH, specific gravity, appearance). Simple chemical screening tests were performed in urine to detect pertinent metabolites, including Benedict test (reducing substances), nitrosonaphthol (tyrosine and metabolites), p-nitroaniline (methylmalonic and ethylmalonic acids), cyano-nitroprusside (cystine and homocystine), cetyltrimethylamonium (glycosaminoglycans), dinitrophenylhydrazine (keto acids) and ninhydrin (amino acids). Creatinine was determined by the Jaffé method. Semi-quantitative analysis of amino acids by circular paper chromatography was performed in urine and plasma. Subsequent procedures were: determination of urinary glycosaminoglycans by cellulose thin layer chromatography (TLC); mono-, di- and oligosaccharide by silica gel TLC; organic acids, by GC-MS; amino acids, by automatic AAA; oligosaccharides by HPLC and cholesterol metabolites by UV-Vis spectrophotometry. In several cases, specific enzyme assays confirmed the suggested diagnosis.

**Results and Discussion**

The Laboratory of Inborn Errors of Metabolism, named LABEIM, at the Department of Biochemistry, Institute of Chemistry, Federal University of Rio de Janeiro (UFRJ) is one of the pioneer diagnostic laboratories for IEM in Brazil; it has been standardizing and developing methodologies since 1988. Eight thousand (8,000) patients from the state of Rio de Janeiro with a high clinical suspicion of having an IEM, the majority children, were analyzed at LABEIM.

Biochemical results obtained from the investigation of 15,000 samples (urine and plasma), evaluated together with the clinical presentation, permitted the identification of IEM in 487 cases (6.1%). The results are summarized in Table 1.

<table>
<thead>
<tr>
<th>Group of diseases</th>
<th>Number of cases</th>
<th>Percentage / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysosomal storage disease (LSD)</td>
<td>220</td>
<td>45.1</td>
</tr>
<tr>
<td>Amino acid metabolism</td>
<td>112</td>
<td>22.8</td>
</tr>
<tr>
<td>Organic acid metabolism</td>
<td>45</td>
<td>9.2</td>
</tr>
<tr>
<td>Carbohydrate metabolism</td>
<td>39</td>
<td>8.1</td>
</tr>
<tr>
<td>Transport defects</td>
<td>29</td>
<td>6.0</td>
</tr>
<tr>
<td>Others (Smith Lemli-Opitz syndrome; Lowe syndrome; vitamine deficiency)</td>
<td>43</td>
<td>8.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>487</strong></td>
<td><strong>100.0</strong></td>
</tr>
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The group of LSD was found to be the most diagnosed in Rio de Janeiro, with mucopolysaccharidoses (MPS) presenting the largest number (142), followed by type II glycogenosis (25) and gangliosidosis GM1 (12).

Lysosomal storage diseases (LSD) comprise a group of disorders involving accumulation of macromolecules, such as glycosaminoglycans, glycoproteins, glycogen and lipids, among others. This accumulation originates from the deficiency of lysosomal enzymes, resulting in lysosome rupture, metabolite accumulation in several organs, and in many cases, increased excretion in urine.

Figure 1 shows the characteristic profile of urinary glycosaminoglycan excretion of MPS type II, besides three normal profiles of patients suspected of having MPS, but without confirmed diagnosis.

Mucopolysaccharidosis type II was the most diagnosed MPS (40 cases) in this survey, and in 2013, Giugliani also reported that this MPS was found to be the most frequent...
in Brazil. The profile of MPS type II is characterized by the presence of the glycosaminoglycans dermatan and heparan, besides the usually excreted chondroitin-4- and -6-sulfates (see the profile in Figure 1, represented by the bands from bottom to top, in sequence). The deficient enzyme in MPS type II is iduronate sulfatase, the first enzyme of the degradation pathways of dermatan sulfate and heparan sulfate, resulting therefore in the accumulation of these metabolites.\(^{18}\)

Several diseases involving glycoprotein, glycogen and lipid metabolism are characterized by the excretion of oligosaccharides (OS). Figure 2 shows OS profiles of three of those diseases: gangliosidosis GM1, one of the most diagnosed in this survey (with a well characterized profile of more intense bands at the beginning of the chromatogram, representing high molecular weight OS); alpha-mannosidosis, a glycoproteinosis (with a characteristic profile of numerous OS bands with various molecular weights), considered rare in Latin America, as to the best of our knowledge no published case was found; Pompe disease, a glycogenosis, under enzymatic replacement therapy (ERT), presenting a band of a glucotetrasaccharide, which is considered a biomarker of this disease.

The total frequency (6.1%) found in our survey covering 8,000 patients is comparable with the ones reported in two articles from the largest laboratory in Brazil, which is reference in Latin America (Hospital de Clínicas de Porto Alegre, Rio Grande do Sul). In 1997 the reported frequency of identified IEM in 10,000 patients was 6.5%\(^{19}\) and in 2001, in 18,000 patients, 8.5%.\(^{20}\) In these surveys, the most diagnosed cases were also LSD.\(^{19,20}\)

The slightly lower frequency found in our survey can be largely attributed to the smaller number of patients, and to differences in laboratory structure. The number of samples that reach LABEIM is limited owing mainly to the few medical genetic services and the absence of a coordinated access from all regions of the state to our laboratory. It must be observed that the reference laboratory in Rio Grande do Sul is highly specialized, owns sophisticated equipment and counts on support from several centers in Europe and USA.\(^{20}\) Implementation of some techniques such as isoelectric focusing (glycosylation defects),\(^{21,22}\) nuclear magnetic resonance spectroscopy (creatine defects)\(^{23}\) and molecular biology analysis (several dysfunctions)\(^1\) would significantly improve the diagnostic capability of LABEIM.

The choice of a single Brazilian laboratory for the comparison with our results is due to the fact that it is the only one presenting published data on a significant number of evaluated patients. Few data on IEM frequency were published previously in Brazil and in Latin America, although interesting findings have been presented in specific congresses and conferences. An example is a study in Argentina, with 14,928 patients (data unpublished in article form) presented at the 11\(^{th}\) Latin American Congress of Genetics, 1994, by the group of the late Argentinian specialist in IEM, Dr. Nestor Chamoiles, which revealed a frequency of 6.25%.\(^{20}\)

At present, IEM are considered a relevant cause of morbidity and mortality among Brazilian children, although
malnutrition, infections, and parasitic infestations still remain the principal causes.

Complete investigation, necessary for a rapid and correct diagnosis of IEM requires sophisticated equipment and high-cost procedures, as well as experienced investigators. In our state, as well as in Brazil, diagnostic centers are still scarce and the ones that exist are unable to diagnose many diseases, due to difficulties such as lack of budget, equipment and adequate infrastructure.24 Fifteen states already have services with some kind of assistance for IEM but biochemical diagnostic laboratories for IEM exist only in six states.25

A larger number of specialized laboratories, as well as experience exchange among them and health services, would increase the knowledge about these dysfunctions, resulting in a growing interest in diagnosis of IEM. Specialized laboratories and multidisciplinary staff, working in both chemical and health areas, would create an ideal condition for diagnosis and treatment of these patients.

Conclusions

This study is an important epidemiological work about IEM in the state of Rio de Janeiro. The group of lysosomal storage diseases was found to be the most recurrent and may represent a reality in this state. Results show that the diseases known as IEM, although considered rare and neglected, affect a significant number of individuals. It would be important to obtain real epidemiological data from the whole country, in order to improve the assistance to the Brazilian IEM risk population. As such, it is essential that the public health system consider the necessity of enlarging the number of specialized diagnostic analytical laboratories and clinical genetic services. A greater support for research projects in this area, besides political will of the authorities, is fundamental.

References


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