The Egyptian Journal of Medical Human Genetics (2014) 15, 379–385

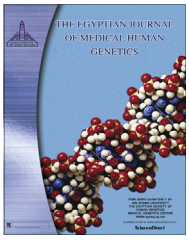
HOSTED BY

ELSEVIER

Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com



ORIGINAL ARTICLE

Fifteen years experience: Egyptian metabolic lab

Ekram M. Fateen, Amr S. Gouda *, Mona M. Ibrahim, Zeinab Y. Abdallah

Biochemical Genetics Department, National Research Centre, Cairo 12311, Egypt

Received 8 June 2014; accepted 2 July 2014
Available online 20 August 2014

KEYWORDS

IEM;
Consanguineous marriages;
Aminoacidopathies;
Lysosomal storage disorders

Abstract *Background:* Inborn errors of metabolism (IEM) are single gene disorders responsible for abnormalities in the synthesis or catabolism of proteins, carbohydrates and fats by means of defective enzymes or transport proteins which results in a block of the metabolic pathway and accumulation of metabolites in different tissues. This study shows the most common diagnosed inherited inborn errors of metabolism among the Egyptian population. Prior to 1995, the diagnosis of inherited metabolic disorders in Egypt was very limited and diagnosed mainly on clinical suspicion. In 1995, The Biochemical Genetics Unit at The National Research Centre has been established as a part of The Human Genetics Department and later on in 2003 it was developed into The Biochemical Genetics Department by applying advanced techniques and equipments and providing early diagnosis for the metabolic disorders which led to better outcome in our patients.

Material and methods: We have retrospectively reviewed a total of 12,148 cases suspected to have inborn errors of metabolism (IEM) with different age groups. They had been referred from several diagnostic centers and hospitals in Egypt to The Department of Biochemical Genetics at The National Research Centre. The diagnosis of these disorders was confirmed by qualitative determination of amino acid profile, quantitative determination of phenylalanine and galactose levels using dried blood spots (DBSs), quantitative determination of urinary glycosaminoglycans (GAGs), two-dimensional electrophoretic separation of GAGs in urine and the assay for lysosomal enzymes activities in plasma and leukocytes.

Results: Out of the total number of cases; 1041 (8.6%) patients were proved to have metabolic disorders. Those patients were classified as: 722 patients (69.4%) with lysosomal storage disorders, 302 patients (29%) with amino acid disorders and 17 patients (1.6%) with galactosemia.

Conclusion: This study illustrates the experience of the reference metabolic lab in Egypt over 15 years. The lab began metabolic disorder screening by using simple diagnostic techniques like thin layer chromatography and colored tests in urine which by time updated and upgraded the methods to diagnose a wide range of disorders. This study shows the most common diagnosed inherited inborn errors of metabolism among the Egyptian population.

1. Introduction

Inherited metabolic diseases are a group of genetic disorders characterized by specific enzymatic defects leading to accumu-

* Corresponding author. Mobile: +20 01001440865.
E-mail addresses: efateen@yahoo.com (E.M. Fateen), amr_gouda3@yahoo.com (A.S. Gouda), monamahmoud60@yahoo.com (M.M. Ibrahim).

Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2014.07.002

lation of metabolites in various tissues and organs resulting in pathologic sequels. The detection of metabolic disorder is done either by measuring the enzyme activities or detection and quantification of the abnormal metabolites by different available techniques.

Aminoacidopathies are a class of inborn errors of metabolism where an enzyme defect inhibits the ability of the body to metabolize certain amino acids. They are autosomal recessive diseases; each of them has a certain characteristic. Early detection of these disorders by quantitation of the amino acids in question leads to early treatment and is either life saving for many newborns or prevents serious sequelae of the disease e.g. mental retardation in case of phenylketonuria (PKU) [1].

Lysosomal storage diseases are a group of inherited metabolic disorders, resulting from a defect in one of the lysosomal catabolic enzymes. They all lead to physical and mental sequelae. Proper diagnosis of these disorders will help to give proper counseling and treatment to the affected siblings and offer prenatal diagnosis to avoid the birth of other affected children in subsequent pregnancies. Also, carrier detection among family members will help proper counseling and avoid birth of further affected children.

Historically, the lysosomal storage diseases were classified on the bases of their storage products, e.g. lipidoses, mucopolysaccharidoses, glycoproteinoses and glycogen storage disease. This categorization brought together diseases with common symptoms reflecting disturbances in the lysosomal catabolic pathway for a particular group of metabolites.

Mucopolysaccharidoses comprise a group of inherited metabolic diseases caused by the deficiency of lysosomal enzymes needed to break down glycosaminoglycans which are long chains of sugar carbohydrates present in cells of bone, cartilage, tendons, corneas, skin and connective tissue. Glycosaminoglycans (formerly called mucopolysaccharides) are also found in the synovial fluid that lubricates our joints. Patients with mucopolysaccharidoses either do not produce enough of one of the 11 enzymes required to break down these sugar chains into proteins and simpler molecules or they produce enzymes that do not function properly. By time, these glycosaminoglycans accumulate in the cells and connective tissues. The result is permanent progressive cellular damage that affects the individual's appearance, physical abilities, organ system functioning and in most cases mental development [2].

Sphingolipidoses are defined as a group of lysosomal disorders leading mainly to organomegaly due to accumulations of sphingolipids in macrophages of the reticuloendothelial system cells. Sphingolipids are found all over the body but are of special importance in the nervous tissue. Galactocerebrosides, sulfatides and sphingomyelins are essential components of the myelin sheath gangliosides. They are found particularly in the grey matter of the brain. Clinical features include progressive psychomotor retardation and neurological problems especially convulsions as well as ataxia. They include: GM1 Gangliosidosis, Tay Sachs, Sandhoff, Metachromatic leukodystrophy, Niemann–Pick disease, Gaucher disease and Krabbe disease [3].

2. Patients and methods

2.1. Patients

This study includes 12,148 cases referred to the Biochemical Genetics laboratory at the National Research Centre (NRC) during the period from January 1995 to December 2010. They were suspected to have metabolic disorders and their ages ranged from 1 day to 20 years. For the determination of amino acid profile, 5–10 ml urine and 1–2 ml heparinized plasma were collected. Blood spots from a heel prick on filter paper were collected for the determination of phenylalanine and total galactose levels in blood. For the determination of level of urinary GAGs and their two-dimensional electrophoretic separation about 5 ml urine was collected. Five ml heparinized whole blood was collected for the separation of white blood cells and measuring activity of the deficient enzymes specific for each disorder fluorometrically. A written informed consent was obtained from all parents of the included patients after full explanation of the study. The ethical approval was obtained from the medical ethics committee at the National Research Center.

2.2. Methods

Methods used throughout this work for the diagnosis of the different metabolic disorders included:

- Qualitative determination of amino acid profile by Thin layer chromatographic separation (TLC) in urine and plasma according to the procedure described by Borden in 1984 [4]. Urine and plasma samples were applied on sheets of cellulose plates on an aluminum foil. The first phase of development included a mobile phase in the form of a mixture of acetone, butanol, acetic acid and water while the second phase had a mobile phase with the same composition in addition to ninhydrin dye.
- Quantitative determination of phenylalanine and total galactose levels in dried blood spot (DBS) according to Slazyk and Hannon [5]: The determination is based on punching a 1/8 inch diameter disc from DBS into polymicrotiter plate, then elution buffer was added and shacked for 30 min. The discs were removed using vacuum manifold. The working reagent was added to each well and shacked for 30 min and then coloring reagent was added and the absorbance was read at dual wave length 570/690 nm.
- Quantitative determination of total urinary glycosaminoglycans (GAGs) according to De Jong et al. [6]: The determination is based on the reaction of GAGs with dimethylmethylene blue dye (DMB) yielding a colored complex that can be measured spectrophotometrically at $\lambda = 520$ nm. GAG concentration (mg/dl) was subsequently normalized to urinary creatinine concentrations to yield final reported values of GAG concentrations in units of mg/mmol creatinine.
- Two-dimensional electrophoretic separation of total urinary GAGs according to Hopwood and Harrison method [7].

Alcian blue was added to centrifuged urine (alcian blue + -GAGs will form complex), followed by the addition of 4 M NaCl, methanol, then 0.1 M sodium carbonate and water. This leads to the dissociation of the complex and formation of alcian blue precipitates. A clear supernatant was transferred to a conical tube, and ethanol was added and centrifuged, then decanted. The precipitate was dried in a small oven and stored at room temperature. Extracted GAGs were applied onto cellulose acetate sheets. In the first run; the buffer used was