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

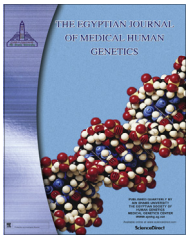
HOSTED BY

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

Pyridine: acetic acid: water. Second run; the buffer used was (0.1 M) Barium acetate. Run time was 3 h. The GAGs were stained by the immersion of the cellulose acetate sheets in alcian blue dye, washed with 5% acetic acid; leaving GAGs present as blue spots.

- Assay of lysosomal enzymes according to methods of Hall et al. [8]:

  Principle of enzyme assay:

  Leucocyte enzymes: Substrate is added to leukocyte homogenate. The tubes are sealed, covered and incubated. The reaction is stopped with a stopping solution. The tubes are shacked briefly and read fluorometrically or calorimetrically according to the used substrate.
  Plasma enzymes: Substrate is added to plasma. The tubes are sealed, covered and incubated. The reaction is stopped with a stopping solution. The tubes are shacked briefly and read fluorometrically.

The work has been carried out in accordance to the Declaration of Helsinki.

## 3. Results

This study included a total number of 12,148 cases suspected to have metabolic disorders with age ranging from 1 day to 20 years and of which 8998 cases (74%) showed positive parental consanguinity (Fig. 1). Of this number, 5985 cases were suspected to have amino acid disorders, 694 cases to have galactosemia and the remaining 5469 cases were suspected to have lysosomal storage diseases. Out of the total enclosed cases, 1041 (8.6%) cases were proved to have a metabolic disorder. 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. Fig. 2 represents the types and percentage of metabolic disorders diagnosed during the period from 1995 to 2010.

## 4. Discussion

Inherited metabolic disorders are a heterogeneous group of genetic conditions mostly diagnosed in childhood and causing substantial morbidity and mortality. This study represents fifteen years experience of biochemical diagnosis for the inherited metabolic disorders at The Biochemical Genetics Department as the main reference lab in Egypt. We have retrospectively reviewed a total of 12,148 cases suspected to have inborn errors of metabolism with different ages who had been referred from several diagnostic centers and hospitals.

This number includes; amino acid disorders, galactosemia and lysosomal storage disorders however organic acidemias were not included since they are recently diagnosed in our lab and were not established fifteen years ago.

Most of the metabolic disorders are autosomal recessive and the incidence of the majority of IEM was high among the included cases. This was due to the high consanguinity rate which was evidenced during collecting data of the subjects and represents about three quarters of the referred cases. Temtamy and Loutife in 1970 stated that the rate of marriage between relatives among the studied Egyptian people was 33% [9] and it was 28.9% in the study done by Hafez et al. in 1983 [10]. In another study performed in 2010 by Temtamy et al., they recorded a rate of about 40% consanguineous marriages [11], while it was 35.3% in the study done by Shawky et al. [12].

Five thousand nine hundred eighty-five were referred to our lab to perform metabolic screening for the amino acids in plasma and urine. The analysis revealed the presence of a large number of cases with generalized aminoaciduria as shown in Table 1 which in most cases result from certain drug administrations such as antibiotics especially penicillin, drugs containing amino acid like N-acetylcysteine, or drugs with sulfur content like 2-mercaptoethanesulfonate or from change in urine pH or from bacterial contamination [13] as their clinical condition did not fit with any of the known aminoacidopathies (Table 2).

### 4.2. Aminoacidopathies

#### 4.2.1. Phenylketonuria (PKU)

Phenylketonuria (PKU) is one of the neurogenetic disorders which was first diagnosed in 1934 [14] and it is considered one of the most common IEM with the highest incidence in

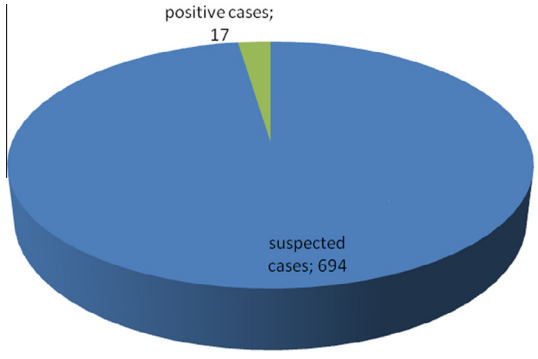



**Figure 1** Carbohydrate disorders diagnosed in The Biochemical Genetics laboratory at The National Research Centre during the period from 1995 to 2010.

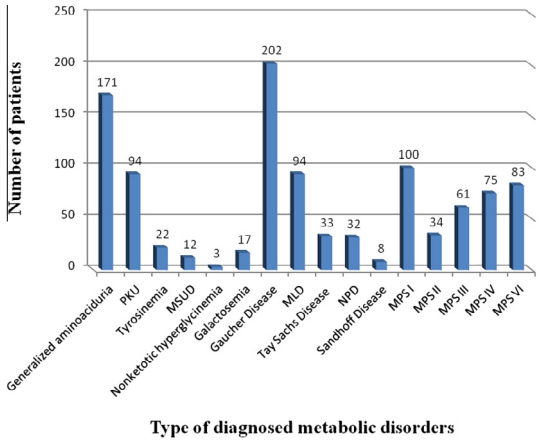



**Figure 2** Type and percentage of 1041 patients with metabolic disorders diagnosed in The Biochemical Genetics Laboratory at The National Research Centre from 1995 to 2010.

**Table 1** Amino acid disorders diagnosed in The Biochemical Genetics laboratory at The National Research Centre during the period from 1995 to 2010.

| Number of suspected cases | Number of positive cases | Amino acid disorders | |
|---|---|---|---|
| 5985 | 302 (5.04% out of 5985) | Generalized aminoaciduria | 171 (56.6%) |
| | | Phenylketonuria (PKU) | 94 (31.1%) |
| | | Tyrosinemia | 22 (7.3%) |
| | | Maple syrup urine disease (MSUD) | 12 (4%) |
| | | Nonketotic hyperglycemia | 3 (1%) |

**Table 2** Lysosomal storage disorders diagnosed in The Biochemical Genetic laboratory at The National Research Centre during the period from 1995 to 2010.

| Number of suspected cases | Number of positive cases | Diseases | | |
|---|---|---|---|---|
| 5469 | 722 (13.2% out of 5469) | Gaucher disease (GD) | | 202 (28%) |
| | | Metachromatic leukodystrophy (MLD) | | 94 (13%) |
| | | Tay sachs disease | | 33 (4.6%) |
| | | Niemann–Pick disease | | 32 (4.4%) |
| | | Sandhoff disease | | 8 (1.1%) |
| | | Mucopolysaccharidoses (MPS): | | 353 (48.9%) |
| | | MPS Type I (Hurler Syndrome) | 100 (28.3%) | |
| | | MPS Type VI (Maroteaux–Lamy) | 83 (23.5%) | |
| | | MPS Type IV (Morquio Syndrome) | 75 (21.3%) | |
| | | MPS Type III (Sanfilippo Syndrome) (22 patients with MPS type III B) | 61 (17.3%) | |
| | | MPS Type II (Hunter Syndrome) | 34 (9.6%) | |

Caucasians 1:10,000 [15]. In patients suspected to have Phenylketonuria simple screening tests in urine such as Ferric chloride ($FeCl_3$) and Dinitrophenylhydrazine (DNPH) tests were done [16]. In the 94 patients proved to have Phenylketonuria, Ferric chloride test in urine gave green color indicating the presence of phenylpyruvic in high concentration and the DNPH test gave positive results in the form of clouding and yellow precipitate indicating the presence of α-ketoacids. By performing thin layer chromatographic separation (TLC) of free amino acids in plasma and urine a distinguishable band of phenylalanine amino acid was clear. The cases were confirmed to have PKU by quantitative estimation of phenylalanine level in dried blood spot [6]. The phenylalanine amino acid levels in blood were found to be elevated in the 94 cases (9% out of 1041 patients with metabolic disorders). The measurement of the amount of the amino acid in blood was a useful tool for monitoring the diagnosed patients who were on diet restriction or on $BH_4$ therapy. About 1/3 of diagnosed cases with aminoacidopathies were confirmed to have PKU which is relatively a high number, so early diagnosis followed by early treatment of phenylketonuria is essential and mass screening programs for the disease are recommended.

#### *4.2.2. Maple syrup urine disease (MSUD)*

Another multi-system degenerative and rapidly progressive IEM is MSUD with a worldwide frequency of 1/185,000 live newborns [17]. It is caused by a defect in the branched chain α-ketoacid dehydrogenase complex. The clinical manifestations are due to the accumulation of both branched-chain amino acids (BCAAs) and branched-chain α-ketoacids [18]. In the included cases, this devastating aminoacidopathy was diagnosed in 12 cases [(12/1041 (1.2%)] which constitutes 4% out of 302 patients proved to have aminoacidopathy. This was done by performing thin layer chromatographic separation of amino acids in plasma and urine which revealed the abnormal abundance of branched chain amino acids (valine, leucine, and isoleucine). In addition to positive DNPH test due to presence of high amounts of ketone bodies [19].

#### *4.2.3. Tyrosinemia*

Hepatorenal tyrosinemia (tyrosinemia type I) is an autosomal recessive inborn error of metabolism due to a deficiency of fumarylacetoacetate hydrolase leading to accumulation of fumarylacetoacetate and succinylacetone. It is usually asymptomatic in newborns, but if left untreated it affects liver, kidney, bone, and peripheral nerves; The incidence of this condition is estimated to be 1:100,000 but may be much higher in certain populations such as the province of Quebec, where it is 1: 20,000 live births [20]. Twenty-two cases [22/1041 (2.1%)] represent 7.3% out of 302 cases diagnosed to have aminoacidopathies. TLC separation of free amino acids in urine and plasma of those patients showed a band of Tyrosine and Nitrosonaphthol test in their urine gave positive results indicating the presence of Tyrosine. In the study of Shawky and Nour El-Din in 2012 [21], they stated that transient neonatal tyrosinemia was detected in 0.05% of cases screened for IEM.

#### *4.2.4. Nonketotic hyperglycinemia (NKH)*

Glycine encephalopathy, which is also known as nonketotic hyperglycinemia (NKH) is a genetic disorder characterized by abnormally high levels of glycine. The worldwide incidence of glycine encephalopathy is unknown. Its frequency has been studied in only a few regions: this condition affects about 1 in 55,000 newborns in Finland and about 1 in 63,000 newborns in

British Columbia, Canada [22]. In this study, only 3 patients (3/1041 0.3%) gave a band of glycine in TLC separation of free amino acids in urine and blood and this represents 1% out of 302 cases diagnosed with aminoacidopathy. In a previous Egyptian study, it was reported that two cases had non-ketotic hyperglycinemia out of a total number of 51 cases confirmed to be positive for IBM during a general screening program in 2001 [23].

Recently we are using more advanced methods to confirm the diagnosis of aminoacidopathies and lipid metabolism disorders. Liquid chromatography mass spectrometry (LC–MS/MS) for performing quantitative estimation of amino acids profile and acylcarnitines in dried blood spots as well as qualitative estimation of organic acids in urine using Gas chromatography mass spectrometry [24]. Early diagnosis and management and early starting of dietary intervention of aminoacidopathies are essential to prevent complications and permanent brain damage and may allow for normal intellectual development. Expanded Tandem MS allows for the detection of elevated amino acids and acylcarnitines concentrations in blood which helps in New Born Screening Program (NBS) for aminoacidopathies as it is recommended in our country.

### 4.3. Galactosemia

Hereditary galactosemia is among the most common carbohydrate metabolism disorders and can be a life-threatening illness during the newborn period. It was first described in a variant patient in 1935 by Mason and Turner [25] who stated that galactose-1-phosphate uridyltransferase (GALT) deficiency is the most common enzyme deficiency that causes increased level of total galactose in blood [26]. Its incidence widely varies as it is 1:70,000 people in the UK, 1:20,000 people in Ireland while in the United States the incidence is approximately 1:40,000–60,000 persons [25]. The disorder is thought to be much less common in Asians. Seventeen patients out of the 694 suspected cases were diagnosed to have Galactosemia (2.44%) by quantitative measurements of total galactose and glactose-1-P in dried blood spots. The early diagnosis and early dietary intervention for galactosemia patients will save the lives of those patients with this treatable metabolic disorder and prevent long term complications so as to reduce the burden on their families and government. Hence, many countries included this disorder in their NBS programs.

### 4.4. Lysosomal storage disorders

Lysosomal storage disorders are genetic disorders in which the lysosomal metabolism of many macromolecules is impaired leading to accumulation of many substances inside the cells leading to their swelling which in turn results in organomegaly with improper functioning of the involved systems. The incidence of their occurrence ranges from 1:5000 to 1:7000 newborns [3]. This class of IEM comprises the defect in metabolism of sphingolipids, mucopolysaccharides, oligosaccharides and lysosomal transport defects [2]. Sphingolipidoses make up about one half of the number of patients diagnosed with lysosomal storage diseases including metachromatic leukodystrophy (MLD), Gaucher disease (GD), Niemann pick disease (NPD), GM2-Gangliosidosis and Krabbe disease [27].

#### 4.4.1. Metachromatic Leukodystrophy (MLD)

It is due to the deficiency of arylsulfatase A enzyme and results in central and peripheral demyelination [28]. This disorder was diagnosed in 94 cases (13% out of the 722 patients diagnosed to have Lysosomal disorders). One hundred and eighteen cases had low in vitro arylsulfatase-A (ASA) enzyme activity and close to the carrier levels but with atypical phenotype of MLD. Those are known as pseudo-deficient cases [29].

#### 4.4.2. Gaucher disease

Gaucher disease results from deficiency of β-glucocerebrosidase enzyme [30]. The disorder has three subtypes with different clinical manifestations. In type I the patient has hepatosplenomegaly and hematological and bone manifestations. In type II the neurological manifestations predominate with rapidly progressive hepatosplenomegaly while in type III the patient has milder neurological involvement [30]. In this study 202 cases [202/1041(19%)] had Gaucher disease which represents 28% out of the 722 cases diagnosed to have lysosomal disorders. They had an age range from infancy to adulthood with a variation in their clinical presentation. High percentage of them was recalled to visit our lab after receiving enzyme replacement therapy in order to measure the activity of chitotriosidase enzyme in plasma for adjustment of the enzyme dosage [31–32]. Diagnosis of this treatable disorder helps those patients to receive their treatment because the government offers the treatment to those patients as it is an expensive one. After receiving the Enzyme Replacement Therapy (ERT) their general condition, hepatosplenomegaly and the hematological manifestations were markedly improved [33].

#### 4.4.3. Niemann–Pick disease (types A and B)

They result from acid sphingomyelinase enzyme deficiency leading to abnormal lysosomal accumulation of sphingomyelin as the main glycosphingolipid product. The number of patients proved to have Niemann–Pick disease was 32 cases. Types A and B of Niemann–Pick disease are mostly differentiated clinically [34]. Niemann–Pick type C syndrome (NPC) has a completely different cause although it has the same name of disorder. In this type the sphingomyelinase enzyme has normal activity and the disease results from a defect in proteins NPC 1 and NPC 2 which are responsible for transporting cholesterol inside and outside the lysosomes respectively. These proteins are encoded by two different genes [35]. NPC is diagnosed by cholesterol study in tissue-cultured fibroblasts [36]. Recently the diagnosis of this type of NP disorder is going to be offered to the suspected patients in our lab.

#### 4.4.4. The GM2-gangliosidoses

These disorders are very rare as the major stored product in lysosomes are GM2-gangliosides [37]. They involve two syndromes: Tay-Sachs and Sandhoff. The former syndrome is due to deficient β-hexosaminidase A and the latter one results from deficiencies in both β-hexosaminidases A and B [38]. In the present study the suspected cases were subjected to assay of β-hexosaminidase A and B enzyme activities in their plasma and 33 (4.6%) of them were diagnosed as having Tay Sacchs while 8 (1.1%) had Sandhoff syndrome.

#### 4.4.5. Krabbe disorder

The number of subjects who were suspected to have Krabbe disorder was the lowest compared to the other IEM among the included cases and this was shown in the results by the absence of Krabbe patients in the diagnosed cases [38].

#### 4.4.6. Mucopolysaccharidoses

Mucopolysaccharidoses are the most commonly diagnosed lysosomal storage disorders among the investigated cases. Each type of MPS has a broad phenotypic spectrum with a large range of the age of onset and severity of manifestations. In the Upper Egypt governorates there were entire families known to have a certain type of MPS that was inherited in their off-springs due to the high rate of consanguineous marriages.

In this study patient groups diagnosed with Hurler Syndrome included one hundred cases. They had abnormally high urinary glycosaminoglycans (GAGs) related to their age and their two-dimensional electrophoretic separation revealed big dermatan sulfate and small heparan sulfate spots. The diagnosis was confirmed by assaying the specific deficient enzyme activity in leukocytes which was completely inactive in severe cases.

Hunter Syndrome (MPS type II) is the only x-linked form of MPS disorders although few girls with that syndrome were recorded [39]. This disease results from a defect in the metabolism of dermatan sulfate and heparan sulfate due to deficient iduronate-2-sulfatase enzyme. Their urinary electrophoretic pattern of separation is characterized by presence of dermatan sulfate and heparan sulfate spots. Measuring the enzyme activity was performed to confirm the diagnosis in 34 patients.

In patients having MPS type III (Sanfilippo Syndrome), signs and symptoms result from a defect in heparan sulfate metabolism due to deficiency in one of four enzymes involved in its metabolism: heparan sulfamidase (MPS type IIIA), N-acetylglucosaminidase (MPS type IIIB), glucosamine-N-acetyltransferase (MPS type IIIC) and N-acetylglucosamine-6-sulfatase (MPS type IIID) [40]. Diagnosed cases with MPS type III were 61(17.3% of the diagnosed patients with MPS) who had a well-defined big heparan sulfate spot separated electrophoretically from their extracted urinary GAGs. Measuring MPS type IIIB deficient enzyme in blood of suspected cases confirmed the diagnosis of 22 patients.

Keratan sulfate is one of the six classes of GAGs that is a major content of connective tissues. Presence of a defect in its metabolism results in MPS type IV A (Morquio Syndrome) [41–42]. The presence of the disease was evidenced in 75 patients by the low activity of galactose-6-sulfatase enzyme.

The number of cases diagnosed with Maroteaux–Lamy Syndrome (MPS type VI) was 83 patients. They had a clinical course similar to that of Hurler Syndrome except that the majority of MPS type VI patients had normal intellectual abilities. The pattern of two-dimensional electrophoretic separation of urinary GAGs showed a big dermatan spot as the defect is in its metabolism. The confirmation of diagnosis was based on the assay of arylsulfatase B enzyme activity in blood which is the defective enzyme in Maroteaux–Lamy Syndrome [43].

The purposes of the laboratory testing in biochemical genetics are to diagnose or exclude inherited metabolic disorders in individual patients where the key metabolite may be quite prominent or to monitor patients who have already been diagnosed and are receiving treatment designed to avoid accumulation of the key metabolites to toxic concentrations while ensuring nutritional adequacy. It is also useful for genetic counseling and prenatal diagnosis for the coming pregnancies and carrier detection [44]. 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 screening 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 included Egyptian patients and NBS programs for the treatable disorders in recommended. Diagnosis of treatable metabolic disorders improved the general condition, avoids dangerous complications and even saves the lives of those patients.

## Conflict of interest

Authors of manuscript declare no conflict of interest.

## References

[1] Walter JH, White FJ. Blood phenylalanine control in adolescents with phenylketonuria. Int J Adolesc Med Health 2004;16:41–5.
[2] Meikle PJ, Hopwood JJ, Clague AE, et al. Prevalence of lysosomal storage disorders. JAMA 1999;281:249–54.
[3] Pinto R, Caseiro C, Lemos M, Lopes L, Fontes A, Ribeiro H, et al. Prevalence of lysosomal storage diseases in Portugal. Eur J Hum Genet 2004;12(2):87–92.
[4] Borden M. Screening for metabolic disease. In: Nyhan WL, editor. Abnormalities in amino acid metabolism in clinical medicine. Norwalk, CT: Appleton, Century, Crofts; 1984. p. 401–17.
[5] Slazyk WE, Hannon WH. Quality assurance in the newborn screening laboratory. In: Therrell BL, editor. Laboratory methods for neonatal screening. Washington, DC: American Public Health Association; 1993. p. 23–46.
[6] De Dong JG, Wevers RA, Laarakkers C, Poorthuis BJ. Dimethylene-blue based procedure for mucopolysaccharidoses. Clin Chem 1989;35:1472–7.
[7] Hopwood JJ, Harrison JR. High resolution electrophoresis of urinary glycosaminoglycans. Anal Biochem 1982;119:120–7.
[8] Hall CW, Liebaers I, Di Vatale P, Neufeld EF. Enzymic diagnosis of the genetic mucopolysaccharide storage disorders. Methods Enzymol 1979;50:439–56.
[9] Temtamy SA, Some genetic Loutife A. Surgical aspects of cleft lip-cleft palate problems in Egypt. CL Palate J 1970:578–81.
[10] Hafez MM, El-Tahan H, Awad Allah MO. Consanguineous meeting in Egyptian population. J Med Genet 1983;20:53–60.
[11] Temtamy SA, Aglan MS, Meguid NA. Genetic disorders in the Egyptians. In: Teebi AS, editor. Genetic disorders among Arab population. Springer; 2010.
[12] Shawky R, El-Awady M, El-Sayed S, Hamadan G. Consanguineous matings among Egyptian population. Egypt J Med Hum Genet 2011;12(2):157–64.
[13] Sanseverino M, Wajner M, Giugliani R. Application of a clinical and laboratory protocol for the investigation of inborn errors of metabolism among critically ill children. J Pediatr 2000;76(5):375–82.
[14] Folling A. Uber ausscheidung von phenylbenztraubensaure in den Harn als stoffwechelanomalie in Verbindung mit Imbezzellitat. Hoppe Seylers Z Physiol Chem 1934;227:169.

[15] Bickel H, Bachmann C, Beckers R, Brandt NJ, Clayton BE, Corrado G, et al. Neonatal mass screening for metabolic disorders: summary of recent sessions of the committee of experts to study inborn metabolic diseases. Public Health Committee Eur J Pediatr 1981;137:133–9.
[16] Renuart AW. Screening for inborn errors of metabolism associated with mental deficiency or neurological disorders or both. N Engl J Med 1966;274:384.
[17] Sofia Q, Laura V, Esmeralda M, Elisa LT, Esmeralda R, Luísa D, et al. Incidence of maple syrup urine disease in Portugal. Mol Genet Metab 2012;100(4):385–7.
[18] Sofia Q, Laura V, Esmeralda M, Elisa LT, Esmeralda R, Luisa D, et al. Incidence of maple syrup urine disease in Portugal. Mol Genet Metab 2010;100:385–7.
[19] Buist NRM. Set of simple side-room urine tests for detection of inborn error of metabolism. Brit Med J 1968;2:745–9.
[20] Thomas HZ, Inderneel S, Amii R, Adedoyin O, Donna J, Eileen F, et al. Newborn screening for hepatorenal tyrosinemia-1 by tandem mass spectrometry using pooled samples: a four-year summary by the New England newborn screening program, 2013.
[21] Shawky R, Nour El-Din S. Profile of genetic disorders prevalent in northeast region of Cairo. EJMHG 2012;13(1):45–62.
[22] Hoover-Fong JE, Shah S, Van Hove JL, Applegarth D, Toone J, Hamosh A. Natural history of nonketotic hyperglycinemia in 65 patients. Neurology 2004;63:1847–53.
[23] Shawky R, Riad M, Osman H, Bahaa N. Screening for some inborn errors of amino acid metabolism which impair mental function. Egypt J Med Hum Genet 2001;2(2):71–91.
[24] Fateen E, Gouda A, Boehles H, Sewell A. Inborn errors of metabolism revealed by organic acid profile analysis in high risk Egyptian patients: six years experience. Egypt J Med Hum Genet 2009;10(2).
[25] Mason HH, Turner ME. Chronic galactosemia: report of case with studies on carbohydrates. Am J Dis Child 1935;50:359–74.
[26] Fridovich-Keil J, Galactosemia Walter. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic and molecular bases of inherited diseases. New York, NY: McGraw-Hill Medical Publishing Division; 2008, 72.
[27] Gieselmann V. Lysosomal storage disease. Biochim Biophys Acta 1995;1270:103–36.
[28] Wraith JE. Lysosomal disorders. Semin Neonatol 2002;7:75–83.
[29] Thomas GH. "Pseudodeficiencies" of lysosomal hydrolases. Am J Hum Genet 1994;54:934–40.
[30] Goker-Alpan OR, Giasson BI, Eblan MJ, Nguyen J, Hurtig HI, Lee VM, et al. Glucocerebrosidase mutations are an important risk factor for Lewy body disorders. Neurology 2006;6:21.
[31] Guo Y, He W, Boer AM, Wevers RA, de Bruijn AM, Groener JE, et al. Elevated plasma chitotriosidase activity in various lysosomal storage disorders. J Inherit Metab Dis 1995;18:717–22.
[32] Beck M. New therapeutic options for lysosomal storage disorders: enzyme replacement, small molecules and gene therapy. Hum Genet 2007;121:1–22.
[33] Khalifa A, Tantawy A, Shawky R, Monir E, Elsayed S, Fateen E, et al. Outcome of enzyme replacement therapy in children with Gaucher disease: the Egyptian experience. EJMHG 2011;12:9–14.
[34] Pavlu-Pereira H, Asfaw B, Poupetova H, Ledvinova J, Sikora J, Vanier MT, et al. Acid sphingomyelinase deficiency. Phenotype variability with prevalence of intermediate phenotype in a series of twenty-five Czech and Slovak patients. A multi-approach study. J Inherit Metab Dis 2005;28:203–27.
[35] Vanier MT. Phenotypic and genetic heterogeneity in Niemann–Pick type C: current knowledge and practical implication. Wien Klin Wochenschr 1997;109:68–73.
[36] Pentchev PG, Comly ME, Kruth HS, Vanier MT, Wenger DA, Patel S, et al. A defect in cholesterol esterification in Niemann–Pick disease (type C) patients. Proc Natl Acad Sci USA 1985;82:8247–51.
[37] Kaback MM. Screening and prevention in Tay-Sachs disease: origins, update, and impact. Adv Genet 2001;44:253–65.
[38] Kolter T, Sandhoff K. Principles of lysosomal membrane digestion: stimulation of sphingolipid degradation by sphingolipid activator proteins and anionic lysosomal lipids. Annu Rev Cell Dev Biol 2005;21:81–103.
[39] Tusch K, Gal A, Paschke E, Kircher S, Bodamer O. Mucopolysaccharidosis type II in females: case report and review of literature. Pediatric Neurol 2004;32:270–2.
[40] Malinowska M, Wilkinson FL, Bennet W, Langford-smith KJ, O'leary HA, Jakobkiewicz-Banecka J, et al. Genistein reduces lysosomal storage in peripheral tissues of mucopolysaccharidosis III B mice. Mol Genet Metabol 2009;98:225–42.
[41] Bank RA, Groner JE, Van Gemund JJ, Maaswinkel PD, Hoben KA, Schut HA, et al. Deficiency in N-acetylgalactosamine-6-sulfate sulfatase results in collagen perturbation in cartilage of Morquio syndrome A patients. Mol Genet Metab 2009;97:196–201.
[42] Wood TC, Harvey K, Beck M, Burin MG, Chien YH, Church HJ, et al. Diagnosing mucopolysaccharidoses IVA. J Inherited Metab Dis 2013;24:245–50.
[43] Jurecka A, Zakharova E, Cimbalistiene L, Gusina N, Kulpanovich A, Golda A, et al. Attenuated phenotype in MPS type-VI (Maroteaux–Lamy) patients carrying the P.R152W mutation. JIMD 2012;35:223.
[44] Lin WD, Lin SP, Wang CH, Hwu WL, Chuang CK, Lin SJ, et al. Genetic analysis of mucopolysaccharidosis type VI in Taiwanese patients. Clin Chim Acta 2008;394:89–93.