Mass spectrometry for screening of metabolic disorders: 9-year biochemical genetics experience

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Background/aim
Inborn errors of metabolism (IEM) are a group of congenital disorders that result from deficiency of enzymes or transporters involved in different metabolic pathways in the human body. The most severe form of these disorders appears early in the neonatal period; however, most types of IEMs are responsive to treatment if started early enough before the appearance of serious complications. The introduction of mass spectrometric techniques for analysis of metabolites accumulated in IEM facilitates the early diagnosis through enabling analysis of a large number of samples in a short period of time using small sample sizes suitable for patients in the neonatal period. The aim of this study was to find out the prevalence of amino acids, fatty acids, and organic acids disorders, using mass spectrometry among Egyptian children with metabolic disorders who were referred to the Biochemical Genetics Lab, Human Genetics, and Genome Research Institute, National Research Centre, Cairo, Egypt, over a period of 9 years.

Patients and methods
The present study enrolled 9245 children who visited Biochemical Genetics Department, Human Genetics, and Genome Research Institute, National Research Centre Cairo, Egypt, during the period from 2013 to 2021. All children were subjected to quantitative analysis of amino acids and acylcarnitine profiles in blood, using liquid chromatography/tandem mass spectrometry, whereas qualitative analysis of organic acids was done in urine by gas chromatography/mass spectrometry.

Results
Of 9245 suspected patients, 552 (5.97%) patients were diagnosed with 13 different types of IEM. A total of 383 (4.1%) patients were diagnosed with aminoacidopathies, 167 (1.8%) patients were diagnosed with organic acidurias, and two (0.02%) patients were diagnosed with fatty acid oxidation disorders. Phenylketonuria is the most prevalent IEM of this study (2%) followed by maple syrup urine disease (0.98%).

Conclusion
The simultaneous analysis of amino acids and acylcarnitines in dried blood spots with analysis of organic acids in urine using mass spectrometry provides an integrated panel for the early detection of IEMs in early years of life, facilitating prompt provision of treatment and avoiding serious complications that can be fatal.

Keywords:
inborn errors of metabolism, mass spectrometry, screening

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Introduction

Inborn errors of metabolism (IEM) are a heterogeneous group of genetic disorders that result from deficiency of a single enzyme, cofactor, or transporter involved in the metabolic pathways affecting proteins, fats, or carbohydrates [1]. The manifestations of the disease occur due to one of three reasons: accumulation of toxic metabolic precursors before the metabolic block, deficiency of metabolically important products, or accumulation of byproducts [2]. Although the individual types of IEM are considered rare, the overall incidence of them is recorded to be exceeding one in 800 [3].

Many IEMs are responsive to treatment, especially when diagnosis is achieved in early stage and treatment is started promptly [4]. The most severe forms of IEMs appear during the first years of life where symptoms are usually nonspecific such as lethargy, poor feeding, seizures, and sometimes coma [5]. Some types of these disorders can manifest the first symptoms later during adolescence. IEM have high

mortality and morbidity rates, which increase with delayed diagnosis.

Children and adolescents having IEM present with a wide range of signs and symptoms, which are not specific to a certain IEM and can also be found in nongenetic disorders. The clinical presentation can range from having a normal physical appearance to severe dysmorphism. The nervous system is the primary system affected in most IEM; however, all the body organs can still be affected, whether the effect is on a single organ or multiple organs are involved. The diagnosis becomes more difficult when multiple organs are affected such as in patients of aminoacidopathies, fatty acid oxidation disorders, and organic aciduria (OA) [6].

The Biochemical Genetics Department at the National Research Center has been leading in the diagnosis of IEMs in Egypt since 1995. It has continuously applied new techniques to diagnose different types of IEM, either by measuring the intermediary molecules resulting due to blockage at a certain point in the metabolic pathway such as in aminoacidopathies and OA or measuring the activity of the deficient enzyme such as in lysosomal storage diseases. The cumulative experience with Egyptian patients showed high incidence of IEMs owing to high consanguinity and emphasized the importance of having screening programs to detect these disorders before appearance of symptoms [7].

Metabolic screening provides an effective tool for the early detection and diagnosis of patients with IEMs, thus improving the prognosis and quality of life. The qualitative analysis of urinary organic acids using gas chromatography/mass spectrometry was established since early 1980s, leading to the detection of disorders of organic acid metabolism [8]. In the early 2000s, the introduction of tandem mass spectrometry in the screening programs facilitated the simultaneous analysis of amino acids and acylcarnitines profiles in a single test with high accuracy and sensitivity.

The aim of this study was to find out the prevalence of amino acids, fatty acids, and organic acids disorders, using mass spectrometry among Egyptian children who were referred to the Biochemical Genetics Lab, Human Genetics, and Genome Research Institute, National Research Center, Cairo, Egypt, over 9 years from 2013 to 2021. The study also aimed to investigate the change in number of diagnosed cases and diagnosis of disorders that could not be detected by old methods after the introduction of mass spectrometry.

Patients and methods

Patients and study design

This was a retrospective study that included 9245 clinically suspected patients referred from several diagnostic centers and hospitals in Egypt to the Biochemical Genetics Department in the Human Genetics and Genome Research Institute, National Research Center, during the period from 2013 to 2021.

Ethical consideration

The present study was conducted based on the Code of Ethics of the World Medical Association and according to the principles expressed in the Declaration of Helsinki. This study was approved by the Medical Research Ethics Committee of the National Research Center with approval number 04055062022, and a written informed consent was taken from the patient's parents or caretakers before their inclusion in the study.

Methods

All patients were subjected to the following:

(1) Quantitative analysis of amino acids and acylcarnitines profiles using liquid chromatography/tandem mass spectrometry (LC-MS/MS).
(2) Qualitative analysis of organic acids in urine by gas chromatography/mass spectrometry.

Quantitative analysis of amino acids and acylcarnitines profiles using liquid chromatography/tandem mass spectrometry

Samples and materials used

A punch of 3.2-mm dried blood spot (equivalent to 3.1 µl of blood) on S&S grade 903 filter paper (Schleicher and Schuell) was used.

Analysis was performed using neonatal Chromsystem kit without derivatization (see brochure for sample preparation). Positive tests were repeated in case of neonates to avoid false-positive results owing to liver immaturity.

Our laboratory participates in the CDC quality assurance program for the quantitative analysis of amino acids and acylcarnitines profiles by tandem mass spectrometry as an external quality control program for accuracy evaluation. According to this program, our laboratory results are compared against the overall mean and the range resulted from using the same neonatal kit of the participating laboratories.

Procedure

All tests were done using Acquity Waters Xevo TQD triple quadrupole mass spectrometer with masslynx, version 4.1, operating software. Electrospray positive ionization mode was selected and parent-to-daughter ion transitions were established after directly infusing each compound to the mass spectrometer. Cone voltage and collision energy were adjusted in multiple reaction monitoring mode to maximize sensitivity and ion intensity.

Qualitative analysis of organic acids in urine by gas chromatography/mass spectrometry

Samples and materials used

Overall, 5 ml of random urine samples was collected from all participants of the study. In some cases, urine samples taken during or shortly after the metabolic crisis were used.

Decanoic acids, heptadecanoic acid, 5 M HCL, diethyl ether, ethylacetate, N,O-Bis(trimethylsilyl) trifluoroacetamide, pyridine, and anhydrous sodium sulfate were all purchased from Sigma-Aldrich (St Louis, Missouri, USA).

Procedure

Extraction and derivatization were done according to the method of Sweetman [8]. In brief, decanoic acid and heptadecanoic acid used as internal standards were added to a volume of urine equivalent to 1 mmol/l creatinine. Liquid–liquid extraction was done using ethylacetate and diethylether. The combined extract was dried by 2 g of anhydrous sodium sulfate and then evaporated under nitrogen gas at 37°C using nitrogen dryer instrument. Then, complete derivatization of samples was done by Bis(trimethylsilyl) trifluoroacetamide.

Analysis was done on gas chromatography electron impact mass spectrometer (Bruker Scion 456 GC – SQ Electron impact MS) with workstation 8 as operating software. Half microliter of the derivatized sample was injected. CP-sil CB capillary column was used. The GC temperature was as follows: injector 250°C, column 90–280°C with an increment of 3°C per min, ion source 150°C and mass analyzer 35°C. The total run time was 75 min.

Statistical analysis

All data were collected, revised, and processed using Microsoft Excel 2007. All data were presented as numbers and percentage.

Results

The present study enrolled 9245 Egyptian children with metabolic disorders who were referred to the Biochemical Genetics Department, NRC, Cairo Egypt, during the period from 2013 to 2021. All patients were distributed into three groups according to their age at referral. First group was up to 6 months, second group from 6 months to 2 years, and the third group from 2 to 5 years. The highest number of patients were in group I (up to 6 months), as shown in Table 1.

Screening of amino acids and acylcarnitines in dried blood spots was done in parallel with screening of urinary organic acids for all patients. The present study reported that out of the 9245 suspected patients included, 552 (5.97%) patients were diagnosed with disorders of different types of IEMs. A total of 383 patients were diagnosed with amino acidopathies (4.1% of referred patients and 69.4% of diagnosed patients), 167 patients were diagnosed with OAs (1.8% of referred patients and 30.3% of diagnosed patients), and two patients were diagnosed with fatty acid oxidation disorders (0.02% of referred patients and 0.4% of diagnosed patients).

In this study, aminoacidopathies included the highest percentage of diagnosed cases, where phenylketonuria (PKU) and maple syrup urine disease (MSUD) were the most prevalent types. OAs presented with lower percentage than aminoacidopathies, where glutaric aciduria type I was the most prevalent among patients diagnosed with OAs. The least percentage was for fatty acid oxidation disorders, where only two cases were diagnosed with HMG coA lyase deficiency. Detailed results are shown in Table 2. This table also demonstrates the main laboratory findings in blood and urine in each diagnosed disorder. For example, elevated levels of phenylalanine and phenylalanine/tyrosine ratio in blood with excretion of phenylpyruvate, phenyllactate, and phenylacetate in urine are key metabolites for diagnosis of PKU, whereas elevations of glutaryl carnitine level in blood with excretion of glutaric acid and 3-hydroxyglutaric acid in urine are key metabolites for diagnosis of glutaric aciduria type I.

Table 1 Distribution of patients according to their age at referral

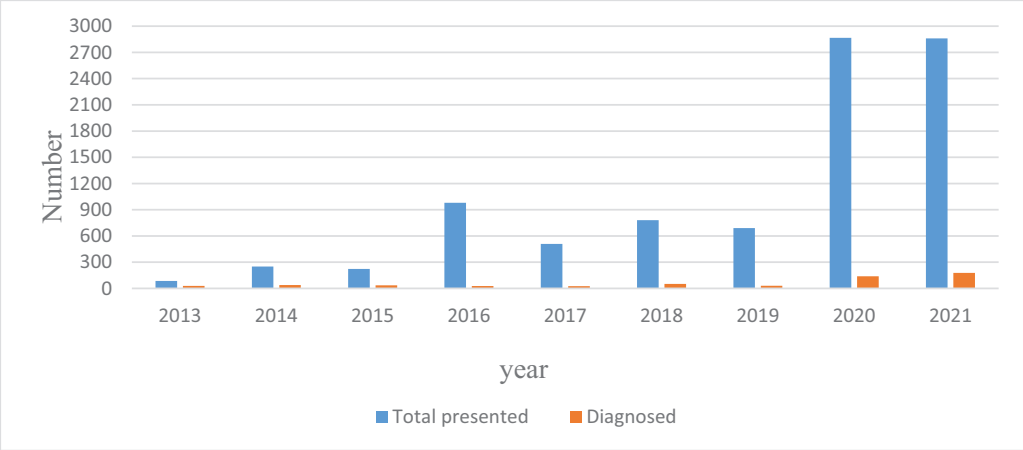
Age group	*n* (%)
0–6 months	5112 (55.3)
6 months–2 years	3210 (34.7)
2–5 years	923 (10)
Total	9245 (100)

Table 2 Types and main laboratory findings of the detected inborn errors of metabolism

Type of IEM	Number of diagnosed patients	% from total patients (*N*=9245)	% from total diagnosed patients (*N*=552)	Main laboratory findings	
				Blood	Urine
Aminoacidopathies					
PKU	198	2	35.9	Phe Elevated phe/tyr ratio	Phenypyruvate Phenyllactate Phenylacetate
MSUD	90	0.98	16.3	Val and leu	2-Hydroxyisovaleric acid 2-Oxoisocaproic acid
Tyrosinemia	62	0.67	11.23	Tyr	4-Hydroxyphenylpyruvic acid 4-Hydroxyphenyllactic acid 4-Hydroxyphenylacetic acid
Nonketotic hyperglycinemia	14	0.15	2.54	Gly	
Citrulinemia	6	0.06	1.09	Citr decreased arg	Orotic acid
Organic acidurias					
Glutaric aciduria type I	60	0.64	10.86	Glutarylcarnitine (C5DC)	Glutaric acid 3-Hydroxyglutaric acid
Lactic aciduria	58	0.63	10.51		Lactic acid
Methylmalonic aciduria	15	0.16	2.71	Gly and ala Propionylcarnitine (c3)	Methylmalonic acid Methylcitric acid 3-Hydroxypropionic acid
Isovaleric aciduria	15	0.16	2.71	Isovalerylcarnitine (C5)	3-Hydroxyisovaleric acid Isovalerylglycine
Propionic aciduria	14	0.15	2.54	Gly and ala Propionylcarnitine (c3)	Metylcitric acid 3-Hydroxypropionic acid
Alkaptonuria	13	0.14	2.36		Homogentisic acid
3-methylcrotonylglycinuria	5	0.05	0.91		3-Methylcrotonylglycine 3-Hydroxyisovaleric acid
Fatty acid oxidation disorders					
HMG-CoA lyase deficiency	2	0.02	0.36	Hydroxyisovalerylcarnitine (C5OH)	3-Hydroxy-3methylglutaric acid Methylglutaconic acid

IEMs, inborn errors of metabolism; MSUD, maple syrup urine disease; PKU, phenylketonuria.

Figure 1



Distribution of the number of referred patients and diagnosed patients over the period of the study.

Figure 1 shows the distribution of the number of patients over the period of the study. It clarifies the gradual increment of both the total referred children and diagnosed cases from the year of 2013 to the end of the study at year of 2021.

The normal levels of all amino acids and acylcarnitines used in this study are shown in Table 3.

Figure 2 shows an example for mass spectrum of amino acids for both a normal participant and a case of

Table 3 Normal levels of different amino acids and acylcarnitines measured in this study

Amino acid	Normal level (μmol/l)	Acylcarnitine	Normal level (μmol/l)
Tyrosine	0–115	C0 (free carnitine)	5–70
Glycine	0–341	C2 (acetyl carnitine)	5–74
Phenylalanine	0–180	C3 (propionyl carnitine)	0–7
Aspartic acid	0–270	C4 (butyryl/isobutyryl carnitine)	0–1.8
Glutamic acid	0–150	C5 (isovaleryl carnitine)	0–1.5
Methionine	0–47	C6 (hexanoyl carnitine)	0–0.5
Citruline	3–40	C8 (octanoyl carnitine)	0–0.32
Leucine/isoleucine	0–216	C10 (decanoyl carnitine)	0–0.37
Alanine	0–439	C12 (Dodecanoyl carnitine)	0–0.6
Phenylalanine/tyrosine	0–0.84	C14 (tetradecanoyl carnitine)	0–1.1
Ornithine	0–163	C16 (hexadecanoyl carnitine)	0.5–9.3
Valine	0–198	C18 (octadecanoyl carnitine)	0–3.5
Proline	0–298	C3DC (malonyl carnitine)	0–0.3
Arginine	0–35	C4DC (methylmalonyl carnitine)	0–2.6
		C5DC (glutaryl carnitine)	0–0.25
		C4OH (hydroxybutyrylcarnitine)	0–1
		C5OH (hydroxyisovalerylcarnitine)	0–0.8
		C5:1 (methylcrotonyl carnitine)	0–0.5
		C10:1 (decenoyl carnitine)	0–0.3
		C14:1 (tetradecenoyl carnitine)	0–0.75
		C14OH (hydroxytetradecanoyl carnitine)	0–0.4
		C16OH (hydroxyhexadecanoyl carnitine)	0–0.3
		C16:1 (hexadecenoyl carnitine)	0–0.6
		C18OH (hydroxyoctadecanoyl carnitine)	0–0.4
		C18:1 (octadecenoyl carnitine)	0–3
		C18:1OH (hydroxyoctadecenoyl carnitine)	0–0.35

tyrosinemia. It demonstrates the difference of relative intensity of tyrosine and its internal standard between normal participant and tyrosinemia case.

Figure 3 shows an example of chromatogram of organic acids for the same tyrosinemia case showing the internal standards peaks (decanoic acid and heptadecanoic acid) and a peak of 4-hydroxyphenyllactic acid (a characteristic accumulated metabolite in tyrosinemia disorder).

Discussion

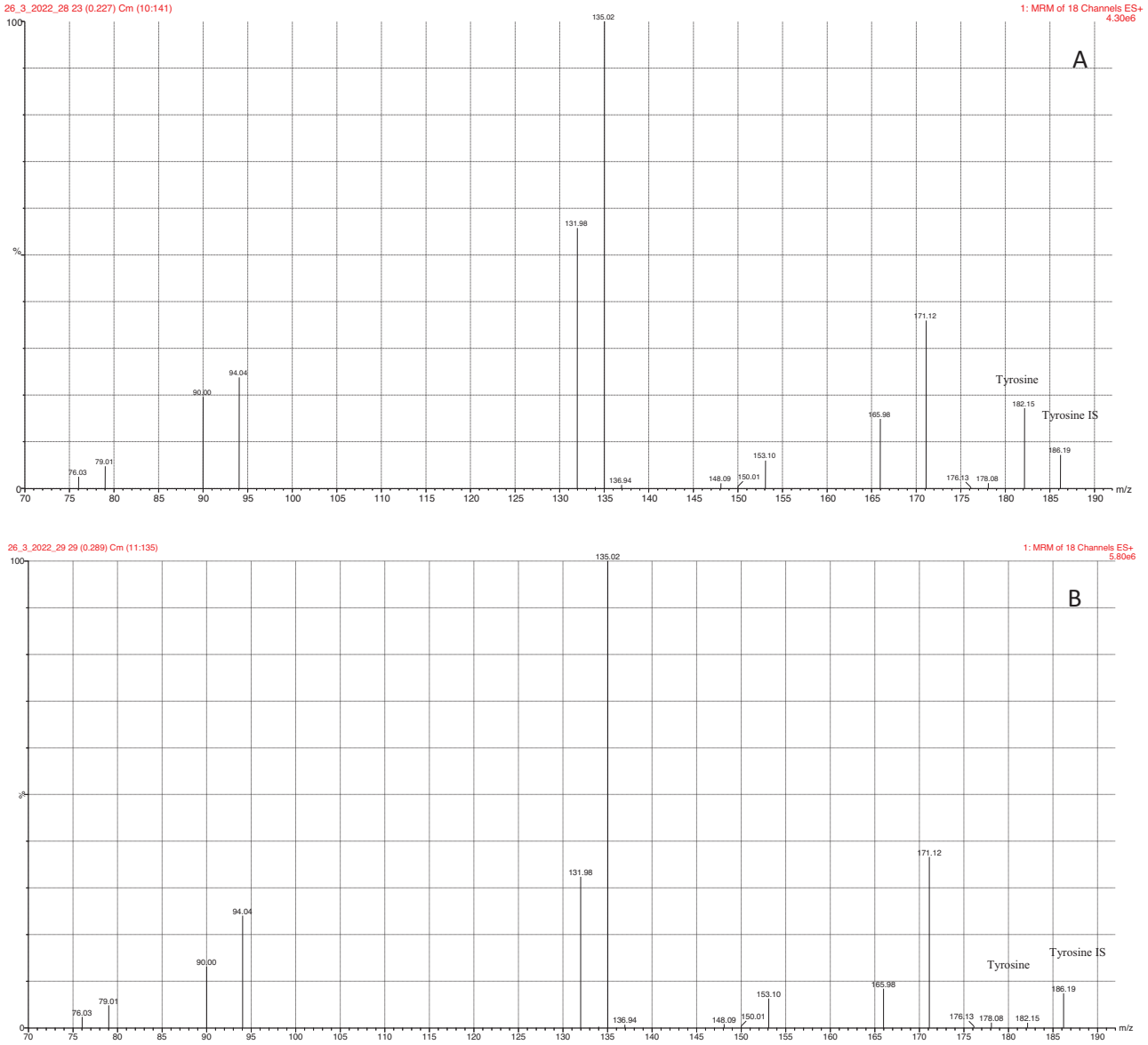
The incidence of IEMs in this study is high when compared with the work done by Wajner and colleagues, who diagnosed 375 patients with different IEMs out of 21 800 highly suspected Brazilian patients (1.7% compared with 5.97% in this study). They could detect 117 patients with aminoacidopathies (0.5% of referred patients) and 258 patients with OAs (1.2% of referred patients). This can be attributed to the significant difference between the consanguineous marriage rates between Brazil and Egypt (4.8% in Brazil compared with 35.3% in Egypt) [9,10].

The prevalence of IEMs in this study is also more than a previous experience in our laboratory before the introduction of mass spectrometric methods for screening of metabolic disorders [7], where only 2.7% of the studied participants had a type of aminoacidopathies and no patient was diagnosed with fatty acid oxidation disorders or OAs. Mass spectrometric methods facilitated the detection of a wider range of amino acids and fatty acids than the previously used thin-layer chromatography method.

The distribution of the number of patients over the period of the study showed a continuous increase of the numbers presented (Fig. 1), with the years 2020 and 2021 having the highest rates of presentation (31% of total patients each). The highest rate of diagnosis was in 2021 (2% of total patients and 32.1% of total diagnosed cases). This increase in the number of patients reflects an increase in the awareness of the importance of metabolic screening in early diagnosis of IEMs.

The classification of patients according to their age at presentation (Table 1) showed group I (0–6 months) as the highest presentation group. Group I is the ideal age of diagnosis of IEM, and if treatment is started at this

Figure 2



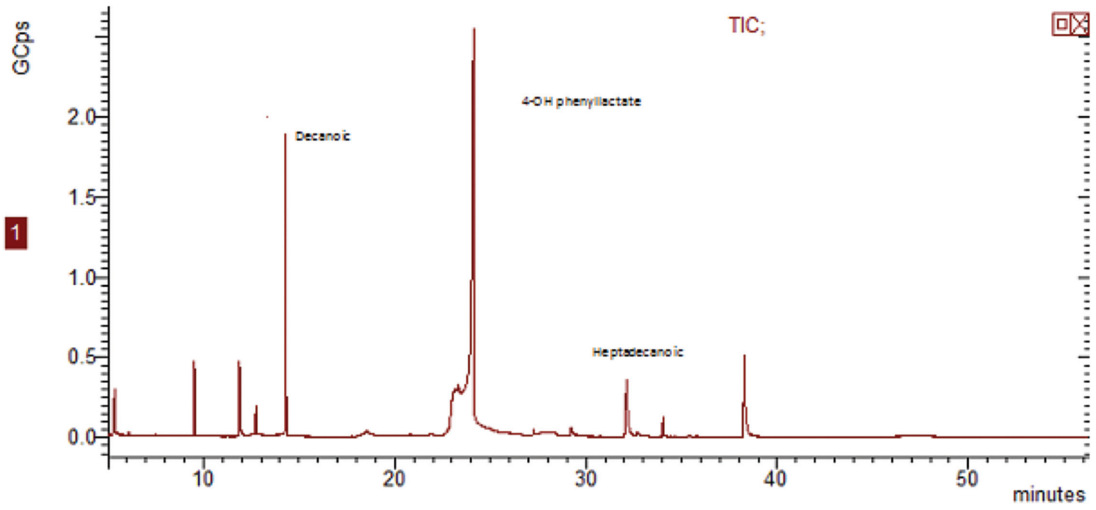
Mass spectrums of amino acids in (a) tyrosinemia patient and (b) normal participant showing the difference in relative intensity of tyrosine to internal standard between patient and normal.

age, a lot of complications can be avoided and mortality rate can be significantly decreased [11]. Mass spectrometric techniques provide the advantage of using a small sample volume suitable for young age as well as the ability of analyzing large numbers of samples simultaneously.

PKU is the most prevalent IEM of this study (2% of total patients). PKU is a part of the national neonatal screening program where it is screened separately from the total amino acid profile [12]. PKU is considered as the most commonly screened IEM owing to its high incidence in different populations. It has been ranked the second most prevalent IEM in Chinese population (1/4391 of newborns) [13] and has been ranked the most prevalent IEM in Egypt in previous studies [7,14]. PKU is a congenital disorder of phenylalanine metabolism that causes severe intellectual disability if left untreated. The early diagnosis and treatment of PKU in the first weeks of life prevents intellectual disability and decreases the incidence of neurophysiological and neuropsychological impairments [15]. All cases of PKU should be further screened for BH4 to determine the underlying cause of the disease.

MSUD is a disorder of branched chain amino acids metabolism owing to deficiency of branched-chain

Figure 3



Gas chromatography chromatogram of tyrosinemia patient showing the peaks of internal standards (decanoic and heptadecanoic) and 4-OH phenyllactate (accumulated metabolite).

alpha-ketoacid dehydrogenase. It was detected in 90 (0.98%) patients of this study. This is a much lower percentage when compared with the study of Khalaf *et al.* [16], who detected MSUD in 9% of their studied patients, and is much higher than the prevalence in a study done by Huang *et al.* [17], who detected MSUD in only 0.05% of their studied patients. Treatment of MSUD is feasible by the dietary restriction of branched chain amino acids, and if it is started early, prognosis is often good [18].

Currently, the Egyptian ministry of health and population is providing screening for PKU and hypothyroidism only. However, based on the prevalence of MSUD in this study, adding MSUD to the Egyptian neonatal screening program can help in detection of a large number of patients and avoid the fatal complications of the disease.

Tyrosinemia was detected in 0.67% of patients of this study and represented 11% of the positive patients. This is a much higher incidence when compared with the study done by Santos *et al.* [9] on Brazilian patients, where they detected only 12 patients with tyrosinemia in a total of 375 patients detected with IEM (3.2%). Unfortunately, in our study, screening for succinylacetone was not provided by the kits used for detection of amino acids and acylcarnitines, and as a result, differentiation of tyrosinemia type I patients was not possible. In addition to the dietary restriction of phenylalanine and tyrosine in all types of tyrosinemia, drug therapy is available for tyrosinemia type I through administration of nitisinone, which prevents the accumulation of toxic metabolites, that is, malylacetoacetate and fumerylacetoacetate produced by the breakdown of the accumulated tyrosine. The outcome of using nitisinone is very good if started within the first few weeks of life [19]. Succinylacetone-measuring kits are better to be used in cases of tyrosinemia.

Nonketotic hyperglycinemia was detected in 0.15% of the patients of this study. This is similar to the numbers obtained by Nagaraja *et al.* [20], while screening high-risk Indian patients. Unfortunately, there is no effective treatment for nonketotic hyperglycinemia, but the administration of sodium benzoate can decrease blood levels of glycine, thus improving some of the symptoms [21].

Citrulinemia is a urea cycle disorder due to deficiency of argininosuccinate synthetase enzyme. It was detected in six (0.06%) patients of the study. This is a high incidence when compared with the study done by Demirelce *et al.* [22] on symptomatic Turkish patients, where they detected only one (0.005%) case of citrulinemia out of 17 066 patients, but is a low incidence when compared with the study by Nagaraja *et al.* [20], who detected it in 0.14% of their studied patients. Citrulinemia starts mainly in the first few weeks of life, and with early dietary restriction and treatment, normal IQ and development can be achieved [23].

OA was detected in 167 patients of this study (1.8% of referred patients and 30.3% of diagnosed patients). The

most prevalent OA was glutaric aciduria type I, which was detected in 0.6% patients of this study and 36% of patients diagnosed with OA. This is very close to an Indian study by Nagaraja *et al*. [20], who detected glutaric aciduria in 0.5% of the patients of their study and 34% of the patients diagnosed with OA. Although both Bahrain and Egypt are considered Middle Eastern countries and both suffer from high rates of consanguineous marriages, Golbahar *et al*. [24] could not detect any case of glutaric aciduria among 1986 symptomatic patients. Early diagnosis of glutaric aciduria type 1 is the gold standard for the effective management of this disease, where the dietary restriction of proteins in addition of carnitine supplementation can keep the patient asymptomatic and prevent the development of neurological disorders [25].

Lactic aciduria was detected in 0.6% of the patients of this study. This is double the prevalence of lactic aciduria in the study done by Wajner *et al.* [4] on Brazilian patients. This increase in prevalence can be explained by the high rate of consanguinity in Egypt when compared with Brazil. Lactic acidosis can be a result of many different enzyme deficiencies, such as deficiencies of glucose-6-phosphatase, fructose-1-6-biphosphatase, pyruvate dehydrogenase, and pyruvate carboxylase. Further investigations should be done to determine the underlying cause of lactic acidosis.

Elevated C3 acylcarnitine as well as the elevation of C3/C2 ratio was detected in 29 (0.3%) patients of this study. The analysis of urinary organic acids of these patients revealed excretion of methylmalonic acid in only 15 patients of them, thus differentiating the patients with methylmalonic aciduria (MMA) from those with propionic aciduria (PA). Heringer *et al*. [26] reported that patients diagnosed through metabolic screening before experiencing decompensation episodes had better movement development and later development of brain injuries than those diagnosed later in their lives.

MMA was detected in only 0.16% of the study patients. It is the sixth most prevalent IEM and third most prevalent OA in this study. This is different from another study done on Egyptian patients [27], in which MMA was detected in 0.6% of the patients of their study. MMA was the most prevalent OA and the second prevalent IEM. The lower incidence of MMA in our study may be attributed to the bigger sample size and the longer period of study, which increased the probability of detecting other IEMs.

PA was detected in 0.15% of the study patients, ranking seventh and fourth in the most prevalent IEM and OA, respectively. This is different from a study done on Saudi patients [28], where PA was the most prevalent disorder among 775 000 screened patients.

Isovaleric aciduria (IVA) is a disorder of leucine metabolism that leads to accumulation of isovalerylcarnitine (C5) in the blood. It was detected in 0.16% of the patients of this study, which is a much higher incidence when compared with the study by Alfadhel *et al.* [28], despite the higher consanguinity rate in Saudi Arabia compared with Egypt. This higher incidence can be attributed to the difference in the criteria of selecting patients, where the Saudi study was based on screening neonates regardless of suspecting IEM. On the contrary, IVA patients represented only 2.7% of the positive cases of this study compared with 3.5% of the positive cases of the Saudi study. The detection of C5 acylcarnitine in blood is not specific for IVA. However, the concomitant detection of isovalerylglycine and three hydroxyisovaleric acid in urine can confirm diagnosis.Alkaptonuria was detected in 13 (0.14%) patients of this study through detection of homogentisic acid in urine. Alkaptonuria can be missed if screening is done in dried blood spot without simultaneous analysis of patients' organic acids in urine.

3-methylcrotonylglycinuria was detected in five (0.9%) patients out of 552 positive patients. This is a lower percentage compared with a previous Egyptian study [29], in which two (2.9%) of 68 positive patients were diagnosed with 3-methylcrotonylglycinuria. This higher percentage can be attributed to the lower number of positive cases detected in the study by Hassan and colleagues and the majority of their patients were taken from neonatal screening not from suspected cases. Our percentage is also much lower than those of Afadhel *et al* [28], who detected the disease in 6.2% of their positive cases. Interestingly, no cases were detected in a study done by Golbahar *et al*. [24] in Bahrain.

HMG-CoA lyase deficiency is the only fatty acid oxidation defect detected in this study. This disease was not detected in many previous Egyptian studies [19,27,29,30]; however, it was previously reported in the Saudi population [28].

Conclusion

Egypt has a very high prevalence of IEM owing to the high rate of consanguineous marriages. Neonatal

screening program in Egypt now includes only PKU and congenital hypothyroidism as routinely screened IEM. However, the high numbers of different IEM detected in this study emphasize the need to widen the spectrum of screening program to include more IEMs, e.g., MSUD, tyrosinemia, GA type I, and lactic acidosis. The early diagnosis and management of these disorders enable achievement of better prognosis and avoidance of serious fatal complications. Screening of organic acids in urine in parallel with screening of amino acids and acylcarnitines in DBS enables detection of other IEMs that cannot be detected by DBS analysis alone, for example, alkaptonuria.

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Conflicts of interest

Competing Interest: The authors declare that they have no competing interests.

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