SELECTIVE SCREENING IN NEONATES SUSPECTED TO HAVE IEMS: A SINGLE CENTER STUDY IN UPPER EGYPT

By

Rofaida M. Magdy*, Heba Salah A. El-Abd, Mohamed Abd El-Aal M. Bakheet*, Tasneem Mohammed Ali***, Montaser Mohamed Mohamed***

*Department of Pediatrics, Faculty of Medicine, Sohag University, Sohag, Egypt

**Genetic Department, Ain Shams University, Egypt

***Department of public health and community medicine, faculty of medicine, Sohag University

Corresponding author: Rofaida M. Magdy

Metabolic and Genetic Unit, Department of Pediatrics, Faculty of Medicine, Sohag University, Sohag, Egypt

Mobile: (+02)01063553250

E-mail: rofidamohamed@med.sohag.edu.eg & rofaidamagdy33@gmail.com

ABSTRACT

Background: *Inborn errors of metabolism (IEM) are individually rare but collectively account for a significant proportion of illnesses. Newborn screening for IEM helps early diagnosis and management, causing a better outcome. However, mass newborn screening (NBS) in Egypt is currently limited to phenylketonuria (PKU) and hypothyroidism. We aimed to investigate the magnitude and pattern of IEM detectable by tandem mass spectrometry (MS/MS) among suspected neonates.*

Methods: *This study included forty-one neonates admitted to the Neonatal Intensive Care Unit (NICU) at Sohag University Hospital between January 2017 and June 2018, where all neonates were suspected of having IEM based on the presence of sepsis-like manifestations, convulsions, altered mental status, persistent metabolic acidosis, persistent hypoglycemia, persistent vomiting, and/or history of sibling deaths. All included neonates underwent thorough history and clinical examination, routine laboratory investigations. Investigations for IEM included Tandem mass spectrometry, plasma ammonia and lactate, urinary organic acids. Brain MRI was individually performed in radiology department in our hospital.*

Results: *13 out of 41 cases were diagnosed to have IEM. The most common diagnosis was maple syrup urine disease in 8 patients with a ratio of 19.5%, followed by medium-chain acyl CoA dehydrogenase deficiency in 3 patients with a ratio of 7.3%. Meanwhile, one patient had short-chain acyl CoA dehydrogenase deficiency and another one patient had isovaleric acidemia with around 2.4%.*

Conclusions: *IEM was identified in nearly a third of selectively screened neonates, which underscores the importance of a high index of suspicion and selective screening for IEM at Egyptian NICUs, particularly with the lack of expanded NBS for IEM in Egypt.*

Keywords: *Inborn errors of metabolism; newborn; early diagnosis; tandem mass spectrometry.*

BACKGROUND

Inborn errors of metabolism (IEM) are quartet of unique disorders initiated by failings of only genes, which protocol for enzymes that expedite the substrata transformation into metabolites **(Fukao and Nakamura, 2019)**. It can cause poor consequences and irreversible damage to different body systems and even death. IEM customarily attend in the neonatal phase or infancy but can appear at any time, even in maturity **(Pasquali and Longo, 2018)**. It is not uncommon as a group of illnesses with a total birth occurrence of 50 per 100000 live births **(Stenton et al., 2020)**.

Ideally, initial pre-symptomatic diagnosis of remediable IEM-disorders for which suitable intermediation is vital. This should be performed to improve the outcome before the metabolic error, which could disrupt the mental or physical health and growth **(El-Hattab et al., 2018)**. Tandem mass spectrometry has high sympathy and specificity. It is used to diagnose more than 30 IEM from a specific dried blood spot (DBS), containing amino, organic, fatty acids' oxidation, and urea phase maladies **(Yang et al., 2018**; **Yang et al., 2020)**, which could be helpful in our current study.

The prevalence of IEM in Egypt is not known. Several studies were dedicated to screening IEM in numerous localized areas **(Shawky et al., 2001**; **Elsobky and Elsayed, 2004**; **Selim et al., 2014** and **Lampret et al., 2015)**. Newborn screening (NBS) helps early detection of certain genetic disorders that are asymptomatic at birth, allowing early intervention to avoid the permanent damage **(Jansen et al., 2021)**. Among Arab countries, only Qatar, Saudi Arabia, and the UAE have nationalized NBS for IEM using tandem mass spectrometry technique **(Saadallah and Rashed, 2007)**. In Egypt, a universal free national newborn screening program is well established for PKU and hypothyroidism. Inversely, comprehensive NBS program for

the other IEM is not available in many countries, including Egypt. A newborn screening program was implemented by the beginning of 2021. It covers all newborns admitted in general governmental hospitals NICUs in all Egyptian governorates. This screening program will include 19 genetic diseases including Congenital hypothyroidism, Congenital adrenal hyperplasia, PKU, BH4 deficiency, Methylmalonic acidemia, Propionic acidemia, Isovaleric academia, Glutaric aciduria type I, MSUD, Tyrosenemia type I, Biotinidase enzyme deficiency, Arginenemia, Ornthine transcarbamylase deficiency, Citrulinemia, Homocystinuria, Galactosemia, Fatty acid oxidation defect, Cystic fibrosis, and G-6-PD deficiency. Therefore, selective screening is considered as a key tool for early detection of IEM cases. The selective screening was performed for exaggerated role with symptoms, clinical indicators, outcomes of routine workroom tests, and/or family record implying a metabolic ailment **(Lampret et al., 2015)**.

The current study aimed to screen neonates IEM-suspected for early detection of IEM among them, and timely starting management to minimalize morbidity and transience in high-risk individuals. We also aimed to investigate the magnitude and pattern of IEM detectable by tandem mass spectrometry among suspected neonates, which could emphasize the importance of expanded early NBS targeting IEM.

PATIENTS AND METHODS

Patients and settings:

This is a prospective study that included 41 neonates IEM-suspected at the NICU of Sohag University Hospital from January 2017 to June 2018. Sample size calculation was done using Epi info program at confidence level of 95% and 5% margin of error. This means 45 or more measurements/surveys are needed to have a confidence level of 95% within ±5% margin of error.

Inclusion criteria:

We included neonates from birth to 28 d who have any of the following parameters: sepsis alike manifestations (lethargy, hypoactivity, poor suckling, and poor crying), spasms, disturbed conscious level, persistent metabolic acidosis, obstinate hypoglycemia, persistent vomiting, or family history of former sibling death without a definite diagnosis.

Exclusion criteria:

We excluded neonates with a definite history of perinatal brain injury, CNS infection, brain trauma, multiple congenital anomalies, and confirmed diagnosis of other causes for the patient manifestations.

Methods:

All cases included in this study were subjected to:

I. History taking:

Focusing on age at presentation, sex, residence, complaint and its duration, prenatal, natal, postnatal history, family pedigree, and developmental assessment.

II. Clinical examination:

Particular attention was given to weight, height and head circumference, general examination, and neurological inspection.

III. Laboratory investigations:

a. Routine laboratory investigations:

Full blood count (CBC) was performed on CELL-DYN 3700, Abbott, laboratories diagnostic Division, IL, USA (normal values: HB 11-17.3 g/dl; WBCs 3.1-21.6 x109/L; platelets 152-472 x 109/L), C-reactive protein (CRP) was performed on Latex agglutination test (normal <6 mg/L), serum electrolytes (Na, K, and Ca) was performed on Medica Corporation's Easylyte analyzer (normal values: Na+: 126-143 mmol/L; K+:4.0-7.9 mmol/L; Ca++:1.1-1.36 mg/dL), blood sugar was performed on Cobas 311 chemistry analyzer system, Roche Diagnostic GMbH, Indianopolis,IN,USA (Normal: 3.5-5.5 mmol/L), and blood gases was done using ABL 800, ABG Radiometer USA (normal values: PH:7.35-7.45; HCO3: 20 + 2 mEq/L; PaCO2: 38-42 mmHg).

b. Specific laboratory investigations:

The amino acids and acylcarnitines analysis of DBS on Guthrie paper were tested using tandem mass spectrometry. The samples were kept in freezer at -20°C till analysis, where most of samples were relayed for analysis within 24 h by EMS to Genetic unit, Ain Shams University (GUASH). The analysis was performed by a tandem mass spectrometer of Waters™ (TQD, USA). The liquid chromatograph is UPLC from Acuity™ (Waters Corporation, Milford, Massachusetts, United States). The tandem mass spectrometry is an ESI quadrupole mass analyzer. Neolynx software (Neolynx Inc., CA, USA) was used for

processing MS/MS data. Urinary organic acids were analyzed by GC/MS using Agilent 7890 and 5975 systems (Agilent Technologies Inc., Santa Clara, California, United States) with HP-5ms. The other investigations, including plasma lactate, and ammonia, were analyzed. Plasma ammonia and lactate were analyzed by Photometer 5010 (model 2016, Germany). Meanwhile, the MRI brain was performed for 16 cases using the method of 1.5-T magnet system (different models and manufacturers) at various imaging centers. Available sequences were axial T2W fast spin echo (repetition/echo times: 3,600–5,540/100–110ms) and T1W spin echo (480–565/14–15) with a slice thickness of 4 to 6 mm; sagittal T1W spin echo and axial fluid attenuation inversion recovery [FLAIR] (6,000–11,000/120–146; inversion time 2,000–2,800ms); coronal T1W spin echo or T2-W fast spin echo; and diffusion-weighted image.

Ethical considerations:

This study was permitted by the Ethics Research Committee of Sohag Faculty of Medicine (Soh-Med-21−7−35). A written updated agreement was achieved from the parents of all neonates included in the report. The data of the study are confidential, and care givers have the right to keep it.

Conflicts of interests: The authors declare that they have no conflicts of interests.

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Statistical analysis:

Collected data were organized and evaluated using SPSS version 25. Quantifiable data were conveyed as mean+standard deviation. Qualitative data were conveyed as the number and ratio of each value where $P<0.05$ was considered significant. The test of significance used to compare qualitative data was chi-square or fisher's exact analysis whenever suitable, while the test of significance used to equate qualitative results was the independent t test.

RESULTS

Table (1): The socio-demographic characteristics of the studied population

Variables		N=41	%
Sex	Male	23	56.1
	Female	18	43.9
Residence	Rural	27	65.9
	Urban	14	34.1
Consanguinity	Positive	36	87.8
	Negative	5	12.2
Gestational age	Full term	40	97.6%
	Preterm	1	2.4%
Age at examination	Mean±SD (range)	7±6 (1-23)	
Age at first symptoms	Mean±SD (range)	4±4 (1-20)	
Sibling health	No sibling death	18	43.9%
	Sibling death with IEM	4	9.8%
	Sibling death (unknown cause)	19	46.3%

* Chi Square, #Independent t test.

As tabulated in **Table 1**, this study included 41 patients, where 23 (56.1%) cases were males, the mean age at admission was (7+6) d. And most of studied cases were from rural areas (65.9%). Previous sibling with IEM was detected in 4 cases: Two had MSUD cases and two had 3-MCC. Meanwhile, no statistically meaningful change ($P<0.05$) was detected regarding age, sex, residence, consanguinity, and sibling health.

Table (2): The main presentation of studied population

Main presentation	N=41
DCL ± convulsions	16 (39%)
Persistent metabolic acidosis	3 (7.3%)
Convulsions	9 (22%)
Previous sibling death	9 (22%)
Sepsis like manifestations	3 (7.3%)
Persistent vomiting	1 (2.4%)
First symptom	N=41
Sepsis like manifestations	12 (29.3%)
Respiratory distress	9 (22%)
Convulsions	10 (24.4%)
Previous sibling death	7 (17%)
DCL	2 (4.9%)
Vomiting	1 (2.4%)

DCL: Disturbed conscious level

Table 2 reported that the disturbed conscious level was the most common presentation found in 16 (39%) cases.

Regarding the first symptom, a high statistically substantial difference (P=0.01) was detected between neonates with IEM and without IEM.

Table (3): Results of the routine laboratory investigations

Variables		N=41
Plasma ammonia	Normal (up to 80 ug/dl)	14 (34.2%)
	Elevated (> 200 ug/dl)	3 (7.3%)
	Border line elevation (> 80-200 ug/dl)	24 (58.5%)
Plasma lactate	Normal (10-16 mg/dl)	14 (24.2%)
	Elevated (>16 mg/dl)	27 (65.8%)
Blood gases	Metabolic acidosis with wide anion gap	15 (36.6%)
Blood glucose	Non-ketotic hypoglycemia	3 (7.3%)
	Ketotic hypoglycemia	1 (2.4%)
	Normal	35 (85.4%)
	Hyperglycemia	2 (4.9%)
Ketones in urine	Positive	8 (19.5%)
	Negative	33 (80.5%)
Organic acids in urine	Normal	14 (24.4%)
	Not done	26 (63.4%)
	Abnormal	1 (2.4%)

The other results, including plasma ammonia, plasma lactate, blood gases, blood glucose, ketones in urine, and organic acids in urine, are disclosed in **Table 3**. A high statistically substantial difference was detected between blood glucose and ketones in the urine (P=0.0006 and 0.001, respectively). Moreover, high statistically significant difference (P=0.01) was detected among organic acids in urine in patients with IEM and others.

Table (4): The result of MS/MS of the studied population

	MS/MS results	N=41
Normal		16 (39%)
Abnormal		17 (41.5%)
	Elevated leucine-isoleucine, leucine:alanine ratio, leucine: phenyle alanine ratio + valine	8 (47%)
	Elevated C4, 6, 8, and 10	3 (17.6%)
	Highly elevated C5 carnitine	1 (5.9%)
	Elevated C4 ,6, and 8	1 (5.9%)
	Elevated C5OH, C4OH	1 (5.9%)
	Elevated ornithine	1 (5.9%)
	Low cirulline	2 (11.8%)
Unremarkable		8 (19.5%)

MS/MS: Tandem mass spectrometry

As found in **Table 4**, abnormal EMS was detected in 17/41 (41.5%) cases. The most common abnormality was elevated leucine-isoleucine, leucine: alanine ratio, leucine: phenylalanine ratio + valine in 8/17 (47%). Based on EMS results, high statistically substantial difference (P=0.000) was unearthed between IEM patients' group and patients without IEM.

Table (5): MRI finding of the studied population

MRI abnormalities	N=16
Bilateral, Symmetrical abnormal high signal diffusely involving cerebellar WM, middle cerebellar peduncles, medulla oblongata, post. aspect of pones and mid brain ,cerebral peduncles, post. Limbs of pones and mid brain, medial aspect of thalami, and both Globus pallidus	8 (50%)
Left hemisphere brain edema	1 (6.25%)
White matter changes	4 (25%)
Diffuse brain atrophy	2 (12.5%)
Swollen basal ganglia	1 (6.25%)

MRI: Magnetic resonance imaging

Additionally, brain MRI was examined for 16 cases, where the most common abnormal finding was detected in MSUD patients 8/16 with a percentage of 50% **(Table 5)**. A statistically significant difference (P=0.02) was detected among MRI-findings in patients with and without IEM.

Table (6): The final diagnosis of studied population

Diagnosis	N=41	Out come
MSUD	8 (19.5%)	5 improved & 3 died
MCAD	3 (7.3%)	1 improved & 2 died
Transient hyperammonemia of newborn	2 (4.9%)	Improved
SCAD	1 (2.4%)	Improved
Isovaleric aciduria	1 (2.4%)	Died
Vitamin B6 dependent seizures	1 (2.4%)	Improved
Not reached	17 (41.5%)	15 discharged & 2 died
Normal	8 (19.5%)	8 discharged

MSUD: Maple syrup urine disease, MCAD: Medium-chain acyl-CoA dehydrogenase deficiency, and SCAD: Short-chain acyl-CoA dehydrogenase deficiency

The IEM was detected in 13/41 (31.7%) of the studied population. In details, and as shown in Table 6, MSUD was the most common IEM detected in 8/41 (19.5%), followed by MCAD detected in 3/41 (7.3%). There are two cases, one full-term and one preterm, showed elevated plasma ammonia levels, which returned to the normal level and considered as transient hyperammonemia of newborns with a ratio of 4.9%. High risk

screening was performed for 4 (9.8%) newborns, where couple of them showed previous sibling with MSUD and two with 3-MCC, and normal results were reported. Normal results were also reported for 4 cases (9.8%) which had family history of 3 previous sibling death and positive consanguinity. These cases acquired clinical, and laboratory follow up and no abnormality was reported. Diagnosis was not reached in 17 cases (61.5%), where 15 cases need advanced investigation as genetic study, and 2 cases died before reaching a diagnosis.

DISCUSSION

In the present study, 41 newborns at our NICU were nominated to operate a choosy vetting for IEM by MS/MS. Among them, 13 (31.7%) cases were identified with IEM. MSUD was the most common identified type of IEM (8 cases), followed by MCAD (3 cases), while only single case had short-chain acyl CoA dehydrogenase deficiency (SCAD), and another had isovaleric acidemia (IVA). The most common presenting manifestations were disturbed conscious level, convulsions, persistent metabolic acidosis, sepsis-like manifestations, and persistent vomiting. This indicates the importance of selective screening and high index of suspicion for IEM among neonates, particularly with the current Egyptian mass newborn screening that is limited only to hypothyroidism and PKU. A newborn screening program was implemented by the beginning of 2021.It covers all newborns admitted in general governmental hospitals NICUs in all Egyptian governorates. This screening program will include 19 genetic diseases including Congenital hypothyroidism, Congenital adrenal hyperplasia, PKU, BH4 deficiency, Methylmalonic acidemia, Propionic acidemia, Isovaleric academia, Glutaric aciduria type I, MSUD, Tyrosenemia type I, Biotinidase enzyme deficiency, Arginenemia, Ornthine transcarbamylase deficiency, Citrulinemia, Homocystinuria, Galactosemia, Fatty acid oxidation defect, Cystic fibrosis and G-6-PD deficiency.

Several studies have been performed in Egypt using selective and mass screening trying to detect IEM cases. Shawky et al. **(Shawky et al., 2001)** reported IEM in 51 (11.3%) out of 450 mentally retarded children. Elsobky et al. **(Elsobky and Elsayed, 2004)** studied 232 suspected cases for IEM,

including 44 neonates, and IEM was detected in 22.7% of them.

In 2015, Shawky et al. **(Shawky et al., 2015)** testified that 13 /40 (32.5%) newborns were presumed to have IEM. In 2019, 200 newborns were vetted for IEM in a commentary guided by Khalaf et al. **(Khalaf et al., 2019)** at Assuit university hospital (NICU unit). IEM was confirmed in 70 (35%) out of the 200 screened neonates. The percentage of identified IEM cases in our study (31.7%) was like that confirmed by Shawky et al. **(Shawky et al., 2015)** and Khalaf et al. **(Khalaf et al., 2019)**, which could be attributed to similar inclusion criteria and age group of patients. However, our results were higher than those reported by Gad et al. **(Gad et al., 2014)** as our study included all admitted neonates in NICU rather than selective screening.

In the Arab countries, some studies were performed to investigate the situation of IEM in newborns. An Omani study reported that 10.8 % of critically ill neonates confirmed to have IEM **(Al Riyami et al., 2012)**. Another study acted in Iraq, including 1758 assumed IEM cases from various age parties. The results showed that 12.7% (244/1758) of suspected cases were diagnosed with IEM **(Arif et al., 2016)**. Regarding other countries, Pishva et al. **(Pishva et al., 2015)** investigated 650 high-risk Iranian neonates and IEM was detected in 94/650 (14%) cases. The rates of IEM confirmed cases reported in the other countries were lower than our study, which may be attributed to a higher rate of consanguinity, and consequently IEM, in our community. However, it should be noted that the generalizability of our research is restricted owing to the slight sample size, being single-centered, and relying on selective screening. Moreover, differences among studies may cause from differences in selection criteria, age groups included in the study, and the advanced laboratory facilities available to reach a diagnosis.

In our study, consanguinity was positive in 36/41 (87.8%) of cases. It was higher than the consanguinity (%) reported by Khalaf et al. **(Khalaf et al., 2019)**, Shawky et al. **(Shawky et al., 2015)**, and Fateen et al. **(Fateen et al., 2014)**. This finding represents the elevated rate of cognate marriage in the region of study.

Most of the studied cases were from rural areas 27/41 (65.9%), as our hospital is located nearby a rural area. High rate of

consanguineous marriage was reported due to cultural background of the rural community. High rates of consanguineous marriage can increase the risk of autosomal recessive disorders, including IEM.

The most common symptom of IEM observed in our study was the disturbed conscious level with or without convulsions in 16/41 (39%) cases, and the first symptom observed was sepsis-like manifestations (lethargy, hypoactivity, poor sucking, and poor wailing) in 12 (29.3%). Our results were like Shawky et al. **(Shawky et al., 2015)** who reported sepsis-like symptoms in 15/40 (37.5%) cases, and Khalaf et al. **(Khalaf et al., 2019)** who reported sepsis-like manifestations as the most common presenting symptom but with higher percentage (122 /200 cases (61%). Comparable findings were recounted by Isabel et al. **(Isabel et al., 2014)** and Vargas et al. **(Vargas et al., 2018)**.

In the modern study, MSUD was the dominant diagnosed type of IEM, representing (53.3%) of total positive cases. Our results were consistent with the results of Wajner et al. **(Wajner et al., 2019)**, in which MSUD was reported in (15.5%) of the total positive cases. While UCD was the most common IEM reported in Khalaf et al. **(Khalaf et al., 2019)** and shawky et al. **(Shawky et al., 2015)** representing (28.5%) and (53.8%) respectively. Galactosemia (29%) was the widespread IEM type reported in an Iranian study **(Pishva et al., 2015)**. The differences among results could be due to the mass screening of presymptomatic neonates in this study, while other studies investigated neonates suspected to have IEM.

In the current study, FAOD was detected in 4/13 cases (3 MCAD &1 SCAD) representing (30.8%) of IEM patients. Our results were similar to those reported by Khalaf et al. **(Khalaf et al., 2019)**, in which FAOD represented 15/70 (21.4%) of positive cases. Two other Egyptian studies showed lower results than our study as they reported FAOD in 7.6% **(Shawky et al., 2015)** and 3.4% **(Selim et al., 2014)** of the tested cases. Our results were higher than Omani and Iraqi studies, which reported FAOD in (16%) and (1.1%), respectively **(Al Riyami et al., 2012**; **Arif et al., 2016)**.

In the present study, organic acidemia was reported in 1/15 (6.7%) patient in the form of isovaleric academia. Shawky et al. **(Shawky et al., 2015)** reported organic acidemia in 3/40 (7.5%)

patients. Khalaf et al. **(Khalaf et al., 2019)** reported organic acidemia in 15/70 cases (21.4%), which was higher than our study. In an Iranian study **(Pishva et al., 2015)**, organic acidemia was reported in 40/92 (43.5%) of total positive cases.

Overdue diagnosis of IEM is linked with numerous serious and irremediable experimental problems, even with starting specific therapies at older ages. On the other side, early diagnosis and management can result in a better outcome **(Selim et al., 2014)**. In Egypt, universal national neonatal screening program is offered for early discovery of hypothyroidism and PKU, but IEM other than PKU are not covered by national screening program. Therefore, selective screening is of great importance in Egypt. Selective screening is done for cases suspected to have IEM with suspicious symptoms, signs, abnormal regular laboratory tests, or family history suggesting a metabolic condition **(Lampret et al., 2015)**. High-risk screening for neonates to families with index cases of IEM is particularly important as it helps to start specific treatment before symptoms develop, which is associated with favorable outcome **(Selim et al., 2014)**. Moreover, early diagnosis is essential for genetic counseling **(Lampret et al., 2015)**.

Cited challenges to implementing expanded NBS for IEM by Tandem mass spectrometry in Egypt include clinical and cost-effectiveness. A study done in the UK reported that NBS for Glutaric aciduria type 1, homocystinuria, isovaleric acidemia, long-chain hydroxyacyl CoA dehydrogenase insufficiency, and MSUD could expand life and save costs quality **(Bessey et al., 2020)**. Another study conducted in Lebanon compared the costs for early diagnosed IEM cases by NBS with the costs of late diagnosis. It was reported that late-diagnosed IEM cases need higher cost than those diagnosed early, as they need to pay more for hospital admission with acute metabolic decompensation, follow up visits, regular laboratory tests, psychometric education, and rehabilitation care. Therefore, NBS for IEM can help save economic resources and decrease the social and psychological stress with less disability and mortality rates. Also, incidental gains in the form of decrease emotive discomfort in caregivers **(Khneisser et al., 2015)**. Carroll et al. and Lee et al. told comparable findings in their reports **(Carroll and Downs, 2006**; **Lee et al., 2014)**. Hence, we

recommend expanding our NBS program to include non-PKU IEM using MS/MS technology because of the above-mentioned benefits. Future Egyptian studies on the cost-effectiveness of expanded NBS for IEM could be useful to support this recommendation.

It is important to note that not all IEM should be included in the NBS program. The conditions covered by NBS programs showed much controversy **(Korenev et al., 2019)**. The Recommended Uniform Screening Panel (RUSP) include a group of disorders advised for widespread screening in the U.S. Medical conditions candidate for neonatal viewing should have certain criteria **(Kellar-Guenther et al., 2020)** including: a) Early detection that helps to improve health outcome; b) Screening tests should have minimum side effects; c) Available, effective, and efficacious screening test; d) Available treatment to be started after positive screening; and e) The ability of screening program to provide feedback to caregivers on positive results reporting.

Tandem mass spectrometry is a good screening means for IEM. It analyzes amino acids and acylcarnitines in blood. Amino and organic acids disorders and fatty acid oxidation defects can be detected at the same time **(Guo et al., 2018)**. The current study has some drawbacks. First, the data were based on selective screening, which does not reflect the prevalence among the general population. Second, the small sample size could limit the full characterization of IEM in our locality and minimize the power of the study to detect statistically significant differences. Third, we relied on MS/MS for diagnosis of IEM without confirmatory genetic or functional enzymatic studies. Last, the study is limited to one medical center with limited generalization.

CONCLUSIONS

Selective screening for IEM diagnosis by MS/MS was performed for 41 neonates at our NICU and identified 13 (31.7%) cases were confirmed to have IEM. MSUD was the most identified IEM (8 cases), followed by MCAD (3 cases), while one case had SCAD, and another had isovaleric acidemia (IVA). Disturbed conscious level, persistent vomiting, convulsions, persistent metabolic acidosis, and sepsis-like manifestations were the most popular warning sign in the confirmed cases. This indicates the importance of selective screening and high index of suspicion for IEM among neonates, particularly with the current Egyptian mass newborn

screening limited to hypothyroidism and PKU. Large-size studies with different diagnosis methods need to be conducted to support this study.

Recommendation

Inborn errors of metabolism should be considered as a differential diagnosis for any critically ill neonate admitted to NICU, specially who has history of previous sibling/s deaths, previous sibling with IEM, convulsions, disturbed consciousness, abnormal reflexes, abnormal muscle tone, wide anion gap metabolic acidosis, hypoglycemia, high plasma ammonia and lactate, and certain MRI finding. Emergency treatment protocols should be initiated as soon as a metabolic disorder is suspected to reduce the mortality rates and poor outcome associated with these diseases. Furthermore, implementation of a national newborn screening for IEM among healthy neonates (mass screening) in Egypt is an urgent need to help early diagnosis and management of this group of disorders as the outcome depends on pre-symptomatic diagnosis and early treatment.

LIMITATIONS

The present study was a single center experience with a small sample size. In addition, the data were based on selective screening, which might not reflect the prevalence among the general population, and the limited time for follow-up might not allow full characterization of the patients' outcomes.

Author contributions: RM designed the study, collected data, and wrote the first draft of manuscript. HE revised study design was involved in data analysis and revised manuscript. MM revised the manuscript. MB supervised the whole study and revised the manuscript. All authors have read and approved the manuscript.

This manuscript has been read and approved by all the authors, the requirements for authorship as stated earlier in this document have been met, and each author believes that the manuscript represents honest work.

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