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Original Article

Tandom Mass Spectrometry in Neonates Suspected to have Inborn Errors of Metabolism

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Abstract

Background: Inborn errors of metabolism (IEM) are a diverse collection of genetic abnormalities that are a significant source of illness and death in children.

Objectives: To determine the prevalence of IEM in newborns with suspected IEM and to diagnosis IEM as soon as feasible to reduce morbidity and death in ill neonates.

Subjects and methods: the study included 50 neonates were admitted to Minia University's Neonatal Intensive Care Unit (NICU) between January 2021 and January 2022 with sepsis-like symptoms (lethargy, hypoactivity, poor suckling, and poor crying), as well as convulsions, persistent metabolic acidosis, persistent vomiting, or a history of previous sib death from an unknown cause, or clinical deterioration in a previously healthy neonate.

Result: Out of 50 neonates, eighteen patients (36%) were diagnosed as having IEM. Urea cycle defect was the commonest IEM diagnosed in 7 (14%) cases, followed by suspected organic academia in 4(8%) cases then MSUD in 2 (4%). TMS confirmed the diagnosis of IEM in 11 out of 18 patients diagnosed with IEM. The other seven cases that had IEM were diagnosed by using other clinical and laboratory investigation. 20 (40%) of the patients had a history of siblings' deaths, whereas 3 (6%) had no history of siblings' deaths (diagnosed with IEM).

Conclusions: IEM is a common cause of neonatal illness in the NICU. IEM studies should be performed regularly in NICUs for babies until national newborn screening can be introduced in Egypt, and they should be done jointly rather than one by one, to maximize patient survival and decrease death and morbidity.

Key words: Tandom Mass Spectrometry; Inborn Error of Metabolism; Neonates

Introduction

Inborn errors of metabolism (IEM) are a highly heterogeneous group of genetic disorders and represent a relevant cause of morbidity and mortality in pediatric population [1].

IEM, which are individually rare but collectively numerous, are well-recognized diseases of the generic class of ‘‘rare’’ diseases. Since first described by Garrod at the beginning of the 20th century, several hundreds of new disorders have been defined, as new biochemical and molecular diagnostic tools became available worldwide [2].

The early diagnosis of IEM by laboratory-based mass screening is an important type of preventive medicine. However, there are several factors that restrict the range of IEM which can be screened for, and number of people to whom it can be made available. Extension of mass screening of neonates for a clinically significant IEM is an ideal desirable strategy. Tandem mass spectrometry (TMS) is a powerful effective diagnostic technique and has been proposed as a means to realize this aim. The main advantages are improved accuracy, sensitivity and specificity over the available existing methods, and its suitability for cost-effective multi-disease IEM mass screening [3].

Since the 1990s, the application of TMS has allowed the expansion of Newborn screening (NBS) programs [4]. Because of its sensitive, specific nature and its powerful ability to analyze dozens of metabolites simultaneously, it can promptly diagnose more of IEM. NBS is an important public health program for improving children's health and is used throughout the world [5].

In 2006, the American College of Medical Genetics (ACMG) declared the guidelines of NBS program and confirmed 29 types of screening disorders [American College of Medical Genetics Newborn Screening Expert [6]. NBS with TMS began in 2001 in China [7]. Currently, an increasing numbers of

NBS centers have carried out this work [8].

Methods

This study was conducted in neonatal intensive care unit of Minia university hospital from January 2021 and January 2022.

The study included neonates admitted to Neonatal Intensive Care Unit (NICU) with sepsis like symptoms (lethargy, hypoactivity, poor suckling, and poor crying), as well as convulsions, persistent metabolic acidosis, persistent vomiting, or history of previous sibling death of unidentified cause, or clinical deterioration in a previously healthy neonate.

Inclusion Criteria: Neonates with sepsis like symptoms (lethargy, hypoactivity, poor suckling, and poor crying), convulsions, persistent metabolic acidosis or persistent vomiting as well as neonates with history of previous sibling death of unidentified cause or clinical deterioration in a previously healthy neonate.

Exclusion criteria were: Neonates with hypoxic ischemic encephalopathy (HIE), neonates with TORSCH infection, neonates with positive blood culture and neonates born to mothers with diabetes mellitus, preeclampsia and hypertension.

Methods: All included patients will be subjected to

Detailed full history with special emphasis on age, sex, Gestational age: Antenatal History: The last normal menstrual period (LMP), past obstetric history and medical history Perinatal history: The child's gestational age (i.e, degree of prematurity) at birth. Presentation of the child and delivery type, birth weight, Apgar score and complications in the neonatal period (eg, intubation time, presence of intracranial hemorrhage on neonatal ultrasonography, feeding difficulties, apnea, bradycardia, infection, and hyperbilirubinemia), symptoms of the patient, age of onset of symptoms, relation of symptoms to feeding, similar cases in the family,

parental consanguinity and previous neonatal death

Clinical examination: General examination of the neonate including: Measurements (length, weight, head, abdominal and chest circumference), vital signs (heart rate, respiratory rate, temperature and blood pressure), general condition and activity and neonatal reflexes (Moro and Suckling), Evaluation of neurological status. laboratory investigations: Complete blood count (CBC); hemoglobin concentration (Hb %), red blood cells (RBCs), white blood cells (WBCs), platelet count, C reactive protein (CRP), electrolytes (Na, K, Ca), blood gases (Po2 , Pco2), PH, Bi-carb, metabolic screening by tandem mass spectrometry (MS/MS) Was done to detect amino acid, acyl carnitine profile and other investigations for IEM including Plasma lactate and Ammonia level Were done for some neonates according to the clinical picture

Ethical approval

Approval of the Research Ethics Committee of the Faculty of Medicine was obtained before the study.

Statistical analysis

Data were checked, entered and analyzed using SPSS version 23 for data processing. The following statistical methods were used for analysis of results of the present study. Data were expressed as number and percentage for qualitative variables and mean $\pm$ standard deviation (SD) for quantitative one.

Results

This retrospective analytical observational study included 50 neonates admitted to Neonatal Intensive Care Unit (NICU) at Minia university hospital for children with sepsis like symptoms (lethargy, hypoactivity, poor suckling, and poor crying), as well as convulsions, persistent metabolic acidosis, persistent vomiting, or history of previous sib death of unidentified cause, or clinical deterioration in a previously healthy neonate from January 2021 and January 2022.

TMS confirmed the diagnosis of IEM in 11 out of 18 patients diagnosed with IEM. Four (8%) patients had high C16-Carnitine, C6-Carnitine, C5-DC, C4-OH (C3-DC) and high C3 DC. The other seven cases that had IEM were diagnosed by using other clinical and laboratory investigation. (Table 1)

Out of 50 neonates, eighteen patients (36%) were diagnosed as having IEM. Urea cycle defect was the commonest IEM diagnosed in 7 (14%) cases, followed by suspected organic academia in 4(8%) cases then MSUD in 2 (4%). Thirty-two patients (64%) had normal screening for IEM. (Table 2)

Regarding presenting symptoms, vomiting and convulsion were significantly higher in cases with IEM compared to cases had not IEM (p=0.001 & 0.012 respectively). Abnormal neurological examination was significantly higher in cases with IEM compared to cases had not IEM (p=0.024). Organic acid in the urine was significantly higher in cases with IEM compared to cases had not IEM (p=0.030). Meanwhile there was no statistically significant differences between the two groups regarding age, gestational age, weight, gender, consanguinity, maturity, jaundice, hepatomegaly previous siblings' death and persistent metabolic acidosis (p>0.05) (Table 3).

Serum ammonia was significantly higher in cases with IEM compared to normal cases (207.14± 117.96 Vs 40.43± 40.75, p=0.007). Organic acid in the urine was significantly higher in cases with IEM compared to normal cases (p=0.030). Meanwhile there was no statistically significant differences between the two groups regarding hemoglobin, WBCs, platelets, AST, ALT, creatinine, ABG with anion gap and serum lactate (p>0.05). (Table 4)

Regarding the outcome, 39 (78%) neonates without IEM were discharged from NICU for follow up, 8 (16%) patients were died and 3 (6%) patients

were discharged as requested by parents and lost follow up. (Table 5)

Laboratory investigations showed that serum ammonia was significantly high in all cases that test done (ranged from 3 to 323 mg/dL). The mean serum lactate was 25.34± 12.21 mg/dL with range from 15 to 78.5 mg/dL. The findings showed acidosis with high anion gap in 7 (14%) cases. Two patients (4%) were suspected to have organic acidemia. (Table 6)

Based on clinical finding, other modalities as a reference standard, TMS can identified IEM in 11 neonates (true positives). While it did not detect IEM in 32 normal neonates (true negatives). Seven patients had false negative TMS results. (Table 7)

Discussion

Newborn screening (NBS) is a public health program for the screening of infants shortly after birth for a list of conditions that are treatable but not clinically evident in the neonatal period that could cause severe illness or death without early detection and treatment [9].

The NBS program aims to diagnose infants born with certain genetic, metabolic and functional disorders [10]. By early diagnosis and treatment, severe neurologic damage can be avoided in advance. NBS is designed to provide early diagnosis and treatment before significant and irreversible damage occurs [11].

As regard the distribution of studied patients as per IEM, we found that 18 patients (36%) were diagnosed as having IEM. Urea cycle defect was the commonest IEM diagnosed in 7 (14%) cases, followed by suspected organic academia in 4(8%) cases then MSUD in 2 (4%). 32 patients (64%) had normal screening for IEM.

However, the study by Shawky et al., [12] reported that 13 patients (32.5%) were diagnosed as having IEM, 7 of them (53.8%) had urea cycle defect, 2 (15.4%) had maple syrup urine disease, while methylmalonic acidemia, fatty acid oxidation defect, mitochondrial disease,

and galactosemia were diagnosed in one patient each (7.7%).

As well, Khalaf et al., [13] found that 70/200 cases (35 %) had confirmed inborn errors of metabolism, and another 8 cases (4%) suspected to have inborn errors of metabolism.

In the study by El-Desouky et al., [14] the frequency of inborn errors of metabolism (IEM) among the studied unapparent causes of FTT cases were 8.6%. Types of IEM diseases among the studied cases were one case for each of biotinidase enzyme deficiency (1.7%), methyl malonic acidemia (1.7%), mitochondrial disease (1.7%), organic acidemia (1.7%) and phenylketonuria (1.7%).

Regarding the distribution of studied patients as per tandem mass spectrometry findings, we revealed the Metabolic screening by tandem mass spectrometry was abnormal in 11 (22%) patients, 4 (8%) patients had high levels of C16-Carnitine, C6-Carnitine, C5-DC, C4-OH (C3-DC) and high C3 DC.

However, the study by Shawky et al., [12] reported that Amino-acid and acyl carnitine profile (metabolic screen) by tandem mass spectrometry was abnormal in 13 patients (32.5%), while organic acids in urine showed methyl malonic acidemia in 1 patient (2.5%).

While Hassan et al., [15] reported that among the 3900 clinically suspected cases, 235 were diagnosed with 20 different IEMs. Amino acid disorders were the most common disease group detected in high-risk patients (145/235 (61.7%)). Organic acid disorders were less common (78/235 (33.2%)), and fatty acid oxidation defects were relatively rare (12/235 (5.1%)). PKU was the most common single IEM detected in high-risk cases (116/235 (49.3%)), compared with 5/13 (38.5%) in NBS cases. Other common diseases in high-risk patients included methylmalonic acidemia (26/235 (11.1%)), MSUD (17/235 (7.2%)), and propionic acedemia (13/235 (5.5%)).

As well, Khalaf et al., [13] found that amino acid disorder (AA) in their study was accounted as the most common disorder detected, and this was in accordance with Selim et al., [16]. Regarding the AA disorders, Khalaf et al., [13] found that urea cycle disorders (UCD) was the most common type in our study, detected in 20/70 (28.5%) of diagnosed cases, with male predominance in 15/20 case (75%). This was in agreement with Shawky et al., [12] as UCD was the most common type with an incidence was 53.8%, and 75% of the diagnosed cases were males. Dissimilar, Gündüz et al., [17] found that UCD was detected only in six cases (11.8%).

Furthermore, in Khalaf et al., [13] study, organic acidemia was presented in 15/70 cases (21.4%), which was higher than in Shawky et al., [12] who detect organic acidemia in 3/40 (7.5%) and in Elsobky, [18] who reported organic acidemia in 1.07%.

Regarding the outcome, 39 (78%) patients were discharged from NICU for follow up, 8 (16%) patients were died and 3 (6%) patients were discharged as requested by parents and lost follow up.

However, study by Shawky et al., reported that 13 patients (32.5%) were diagnosed as having IEM, out of these patients, 12 patients (30%) were discharged from NICU after therapy, and one patient (2.5%) died (the one who had mitochondrial disease).

While the study by Khalaf et al., [13] reported that the outcome of the cases with IEM showed that 36% of them were discharged on treatment and continue follow-up at genetic unit and the rest of the cases (64%) died due to complications.

Finally, Laboratory investigations showed that serum ammonia was significantly high in all cases that test done (ranged from 3 to 323 mg/dL). The mean serum lactate was 25.34± 12.21 mg/dL with range from 15 to 78.5 mg/dL. The findings showed acidosis

with high anion gap in 7 (14%) cases. Two patients (4%) were suspected to have organic acidemia; as hyperammonemia is usually triggered by protein catabolism caused by prolonged fasting, fever, infections, gastrointestinal bleeding, dehydration, high protein intake, anesthesia and surgery [19]. In the current study the main presentation of them was Vomiting, poor feeding and acidosis this is the reason why the serum ammonia was significantly high in all cases.

Khalessi et al., [20] report perinatal asphyxia as a cause of hyperammonemia, because of the hypoxic stress, which can induce an increasing catabolism with a decrease in hepatic urea synthesis, leading to mild hyperammonemia. In their results among 100 patients with perinatal asphyxia, 20% patients had hyperammonemia above 90 µmol/L, with a mean plasma level of 117±41 µmol/L in asphyxia stages 2 and 3

Our results were supported by study by Shawky et al., [13] reported that the Laboratory investigations showed that serum ammonia was significantly high in all cases (ranged from 200 to 330 mcg/dL), with no acidosis, and normal extended metabolic screening test. Serum ammonia was high in 32.5 % of the cases and high Plasma lactate was found in 87.5% of the studied group.

Conclusions

From this study we conclude that IEM represent a significant cause for sick neonates in NICU, and it should be considered in the differential diagnosis of any sick neonate. Investigations for IEM should be done routinely in NICU for neonates, until nationwide newborn screening can be applied in Egypt, and they should be done collectively not one by one in order to improve survival and decrease mortality and morbidity of patients.

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Author's contributions

MS and AE conceptualized the work and designed the study, participated in data analysis, interpretation of data as well as drafting of the article. AA conceptualization and study design, data collection, interpretation of data, revision of draft critically for important intellectual content; and final approval of the version to be published. MA conceptualization and study design, interpretation of laboratory investigations, drafting the article and final approval of the version to be published. All authors shared in revision of draft critically for important intellectual content and final approval of the version to be published

Conflict of interest

The authors declare that they have no competing interests

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Table (1): Distribution of studied neonates as per tandem mass spectrometry findings

Parameters			**Studied neonates (n= 50)**	
			n	**%**
TMS	**No specific findings (Normal)**		39	78.0%
	Abnormal (n= 11)	**High leucine,isoleucine, valine, leucine:phenylalanine ratio and leucine:alanine ratio**	2	4.0%
		High free carnitine	1	2.0%
		High propionyl carnitine (C3), and C3:C2 ratio	1	2.0%
		MCAD deficiency (High C6,C8,C10)	1	2.0%
		High citrulline and methionine	ort1	2.0%
		High C16-Carnitine , C6-Carnitine , C5-DC, C4-OH (C3-DC) ,and high C3 DC	4	8.0%
		High tyrosine level	1	2.0%

n: number, %: percentage, IEM: Inborn errors of metabolism, TMS: tandem mass spectrometry, MCAD: Medium-Chain Acyl-CoA Dehydrogenase Deficiency

Table (2): Distribution of studied neonates as per IEM disorders

	Parameters	**Studied neonates (n= 50)**	
		n	**%**
IEM	**Normal screening for IEM**	32	64.0%
	Urea cycle defect	7	14.0%
	MSUD	2	4.0%
	Methyl melanoic acidemia	1	2.0%
	Fatty acid oxidation defect	1	2.0%
	Confirmed organic academia	4	8.0%
	Confirmed tyrosinemia	1	2.0%
	Confirmed mitochondrial disorder	2	4.0%

n: number, %: percentage, IEM: Inborn errors of metabolism, MSUD: Maple syrup urine disease.

Table (3): Comparison between neonates with and without IEM cases regarding demographic and clinical data

Variable		Neonates without IEM (N=32)		Neonates with IEM (N=18)		Test value	P-value
		No.	%	No.	%		
Age (days)	**Mean± SD**	13.19±6.91		15.17±5.36		T= 1.05	0.300
	Median	13.0		16.0			
	Range	3.0- 25.0		4.0- 25.0			
Gestational Age (weeks)	**Mean± SD**	37.97± 1.12		36.2± 2.25		$^{Z}_{MWU}$= 0.628	0.530
	Median	38.0		36.0			
	Range	32.0-39.0		32.0-39.0			
Weight (Kg)	**Mean± SD**	2.54± 0.75		2.88± 0.62		T= 1.93	0.110
	Median	2.56		3.06			
	Range	1.26- 3.67		1.49- 3.62			
Head circumference (cm)	**Mean± SD**	34.5± 2.54		34.25± 8.25		^{Z}MWU= 0.097	0.923
	Median	34.50		33.0			
	Range	29.0-36.0		29.0-36.0			
Gender	**Male**	25	78.1%	15	83.3%	X^2= 0.195	0.659
	Female	7	21.9%	3	16.7%		
Consanguinity	**No**	21	65.6%	8	44.4%	X^2= 2.12	0.145
	Yes	11	34.4%	10	55.6%		
Maturity	**Full term**	26	81.3%	15	83.3%	X^2= 0.0.34	0.854
	Preterm	6	18.8%	3	16.7%		
Presenting symptoms	**Jaundice**	0	0.0%	1	5.6%	X^2= 0.271	1.00
	Hepatomegaly	0	0.0%	1	5.6%	X^2= 0.271	1.00
	Vomiting	0	0.0%	7	38.9%	X^2= 11.42	**0.001**
	Convulsions	3	9.4%	8	44.4%	X^2= 6.34	**0.012**
	previous siblings' death	2	6.3%	1	5.6%	X^2= 0.271	0.602
	Persistent metabolic acidosis	3	9.4%	6	33.3%	X^2= 3.004	0.083
Neurological examination	**Normal**	15	46.9%	2	11.1%	X^2= 5.07	**0.024**
	Abnormal	17	53.1%	16	88.9%		

P value< 0.05 is significant, P value< 0.01 is highly significant, SD: Standard deviation, T= Student T test
ZMWU = Mann- Whitney U test, X^2= Chi- Square test

Table (4): Comparison between neonates with and without IEM cases regarding laboratory data

Variable		Neonates without IEM (N=32)		Neonates with IEM (N=18)		Test value	P-value
Hb (gm %)	**Mean± SD**	18.8± 0.9		18.3± 0.7		Z_{MWU} = 1.04	0.341
	Range	15.2- 19.2		16.9 – 19.3			
WBCs (cm^3× 10^9/L)	**Mean± SD**	16.4± 7.9		14.1± 4.7		T= 1.39	0.172
	Range	2.7- 28.0		4.5- 21.5			
Platelets (× 10^3/mcL)	**Mean± SD**	213.2± 52.5		227.4± 61.6		T= 0.966	0.338
	Range	84.0 - 347.0		85.0- 376.0			
AST	**Mean± SD**	31.25± 13.68		27.75± 5.28		Z_{MWU} = 1.56	0.118
	Range	17.0 - 140.0		21.0 - 38.0			
ALT	**Mean± SD**	17.06± 8.77		14.60± 6.82		Z_{MWU} = 1.08	0.279
	Range	5.0 – 70.0		6.0 – 26.0			
Urea	**Mean± SD**	14.93± 2.51		14.15± 2.5		Z_{MWU} = 1.29	0.197
	Range	10.0 – 19.0		11.0 – 19.0			
Creatinine	**Mean± SD**	0.72± 0.15		1.10± 1.96		Z_{MWU} = 0.758	0.449
	Range	0.30 – 1.00		0.30 – 9.40			
Serum ammonia	**Mean± SD**	40.43± 40.75		207.1± 117.9		Z_{MWU}= 2.69	0.007
	Range	3.0- 98.0		9.0- 313.0			
Serum lactate	**Mean± SD**	23.26± 3.88		30.31± 21.41		Z_{MWU}= 0.143	0.886
	Range	15.0- 30.0		15.0- 78.50			
ABG with anion gap	**Normal**	17	53.1%	9	50.0%	X^2= 4.98	0.083
	Acidosis with high anion gap	2	6.3%	5	27.8%		
	Acidosis	13	40.6%	4	22.2%		
Organic acid in the urine	**Not done**	31	96.9%	12	66.7%	X^2= 8.98	0.030
	Normal	1	3.1%	4	22.2%		
	High methyl malonic acid	0	0.0%	1	5.6%		
	Non-glucose reducing substance and G-1-P	0	0.0%	1	5.6%		

P value< 0.05 is significant, P value< 0.01 is highly significant, SD: Standard deviation, T= Student T test
ZMWU = Mann- Whitney U test, X^2= Chi- Square test

Table (5): Distribution of studied neonates as per outcome

Parameters		Studied neonates (n= 50)	
		n	**%**
Outcome	**Discharged from NICU for follow up**	39	78.0%
	Died	8	16.0%
	Discharged as requested by parents and lost follow up	3	6.0%

n: number, %: percentage,

Table (6): Distribution of investigations of inborn errors of metabolism of the studied neonates

Parameters		Studied neonates	
		n	**%**
Serum ammonia (mg/dL) (n= 21/50)	**Mean± SD**	151.57± 126.6	
	Median	98.0	
	Range	3.0-313.0	
serum lactate (mg/dL) (n= 21/50)	**Mean± SD**	25.34± 12.21	
	Median	24.0	
	Range	15.0-78.5	
ABG with anion gap (n=50)	**Normal**	26	52.0%
	acidosis with high anion gap	7	14.0%
	Acidosis	17	34.0%
Organic acid in the urine (n= 7/50)	**Not done**	43	86.0%
	Normal	5	10.0%
	high methyl malonic acid	1	2.0%
	non-glucose reducing substance and galactose 1 phosphate	1	2.0%

Table (7): Validity of TMS in diagnosis of IEM in studied neonates

Item	IEM Diagnosis by Clinical finding, other modalities confirmed			
	No (n = 32)		Yes (n = 18)	
	n	%	n	%
IEM Diagnosis by TMS				
No (n= 39)	32	0%	7	9.5%
Yes (n= 11)	0	100%	11	90.5%

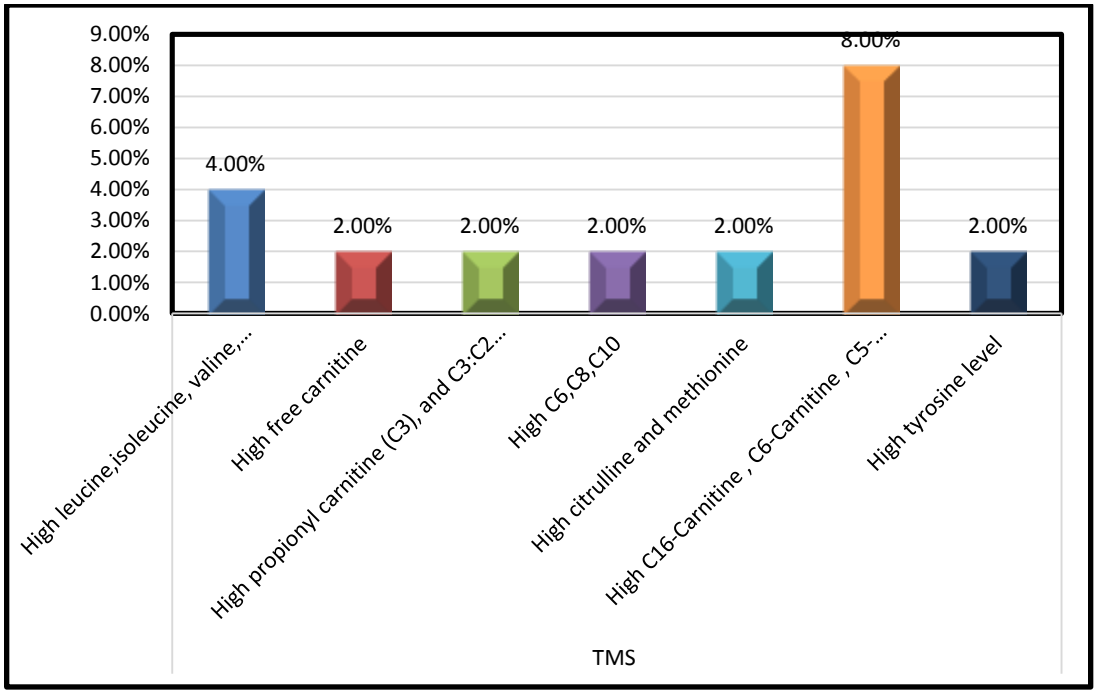

Figure (1): Distribution of the studied cases regarding tandem mass spectrometry findings.

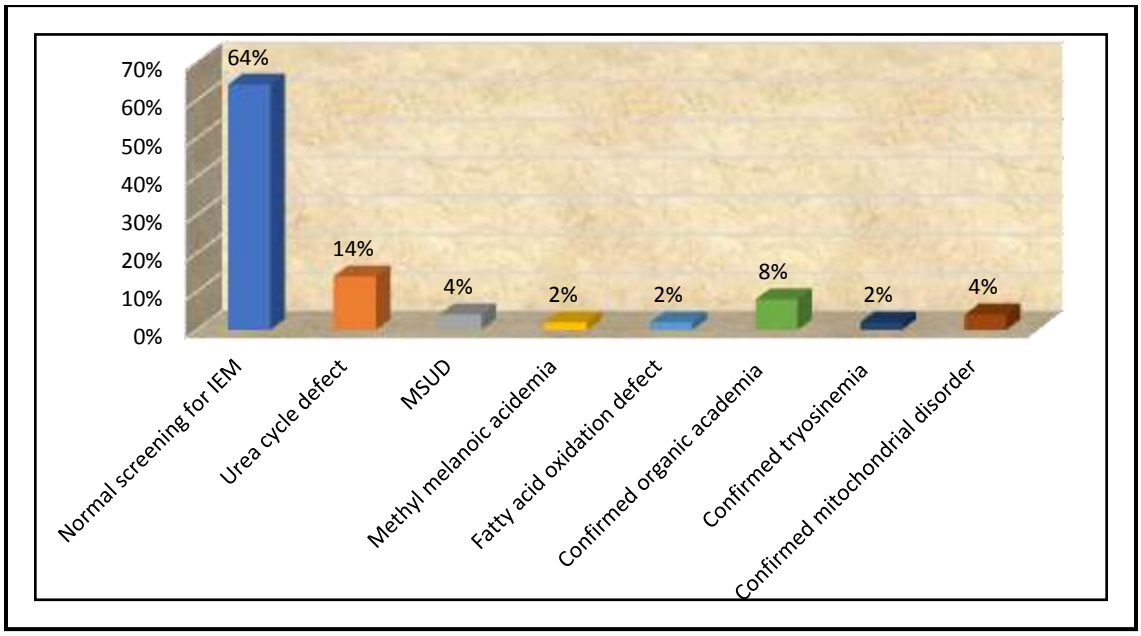

Figure (2): Distribution of the studied neonates regarding IEM disorders.

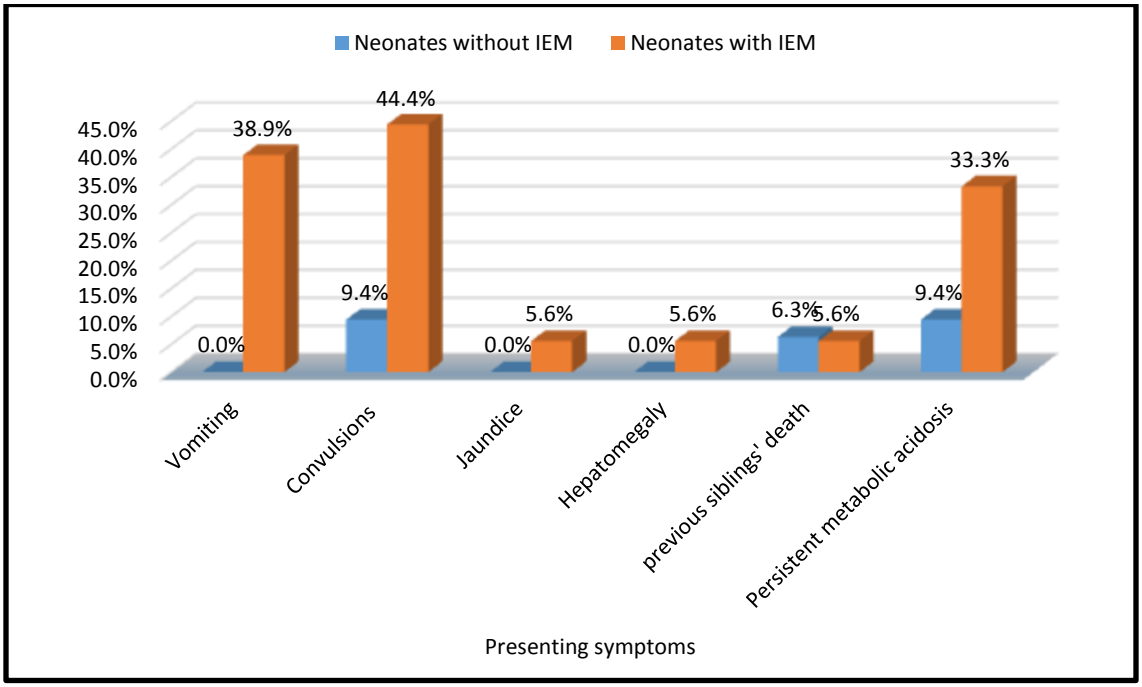

Figure (3): Comparison between the studied groups regarding presenting symptoms.

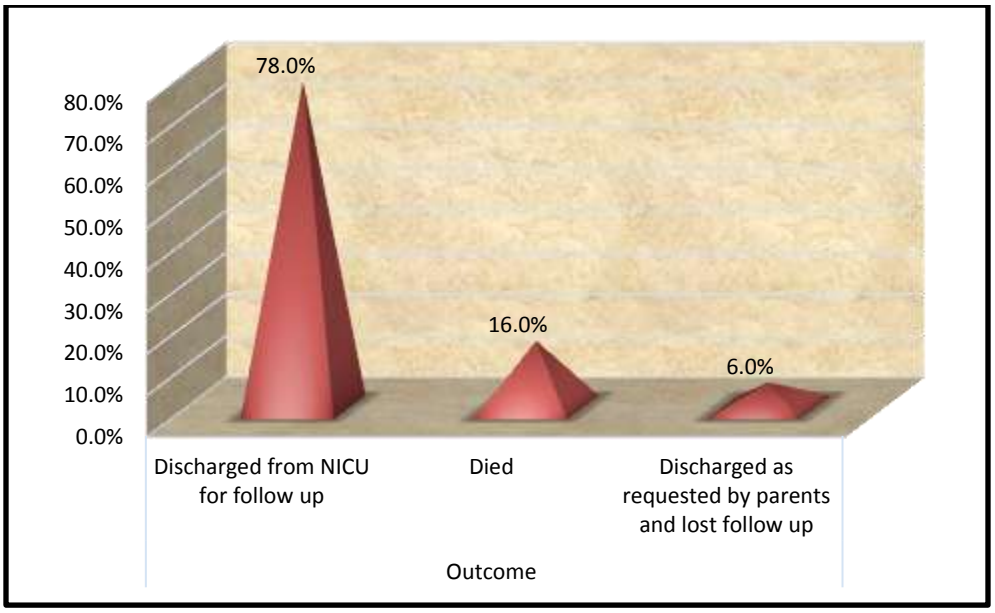

Figure (4): Distribution of the studied neonates regarding outcome.

Citation: Mahmoud M. Shames El-dine; Abdel-Azeem M. El-Mazary; Moustafa A. Abo-Elela; Ashraf M. Abdel-Fadil. "Tandom Mass Spectrometry in Neonates Suspected to have Inborn Errors of Metabolism". *Annals of Neonatology Journal* 2022; 4(2):129-147 doi: 10.21608/anj.2022.121894.1045