DEATH DUE TO INBORN ERRORS OF METABOLISM IN CHILDREN LESS THAN 5 YEARS OLD IN MALAYSIA

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ABSTRACT

This study was conducted to investigate common inborn errors of metabolism (IEM) as the cause of sudden unexplained death in Malaysian children less than 5 years old using tandem mass spectrometry (TMS) as the screening platform. A two-year cross-sectional study from December 2012 to December 2014 were conducted in five government hospitals in Malaysia and Institute for Medical Research Kuala Lumpur. A total of 138 cases were enrolled into the study after satisfying the inclusion and exclusion criteria. In general, 138 dried blood spot, 59 plasma, 59 whole blood and 4 urine samples were received. The laboratory results were analysed by Microsoft excel and SPSS version 21. All statistical analysis was considered significant when $p<.05$. We detected mutations in two cases of IEM by molecular analysis: urea cycle defect (Ornithine transcarbamylase deficiency) and mitochondrial trifunctional protein (MTP) deficiency. Appropriate cut-offs for post-mortem samples allow detection of common IEM in children with sudden death. In combination with molecular genetics accurate post-mortem diagnosis for IEM can be made. Screening by TMS for common IEM should be carried out for all children presented with sudden death.

KEYWORDS: Dried blood spot (DBS), inborn errors of metabolism (IEM), sudden death, tandem mass spectrometry (TMS)

INTRODUCTION

Sudden unexpected death in infancy (SUDI) encompasses all sudden and unexpected deaths in the first year of life whereas sudden infant death syndrome (SIDS) is a term that has been used to describe unexpected death of infants when subsequent investigations failed to demonstrate a definite cause of death (Krous 2004). A small percentage of SUDI cases are attributed to inborn errors of metabolism (IEM). Detection of the cause of death is vital in an effort for prevention of morbidity and mortality amongst the siblings. Furthermore, some of the cases can be misinterpreted as being due to non-accidental injury. Without the appropriate samples and laboratory investigations, deaths attributable to IEM may be undiagnosed at autopsy. A comprehensive laboratory investigation enables accurate clinical diagnosis to be made and thus facilitating timely counselling to the affected families. A coordinated plan of investigation should include accurate details of clinical and family history. A detail external examination may reveal dysmorphism as the external feature of some IEM. The collection of appropriate samples at post-mortem is of paramount importance in excluding IEM as cause of death (Olpin 2004). Biochemical procedures have often been applied to investigate fatalities without definite pathological evidence, including hypothermia, hyperthermia, electrocution, asphyxiation, drowning, uraemia, and acute cardiac death. However, the procedures are also useful for investigating the death process to support and screening for morphologically unexpected causes of death in routine casework (Maeda et al. 2009). The use of post-mortem biochemistry and molecular biology has the advantages of investigating systemic pathophysiological functional changes involve ed in the dying process (Maeda et al. 2009). However, despite the important contribution to a full autopsy, diagnosis of IEM in post-mortem samples is challenging due to the matrix changes that occur in the body upon death. Metabolic profiles in body fluids reflect endogenous changes while the altered metabolite profiles reflect changes that occur following death (Donaldson and Lamont 2013). Hence a different cut-off needs to be established from the post-mortem samples.

The results of most studies done on the occurrence of IEM in children with sudden death had shown that fatty acid oxidation and organic acid disorders are the major causes of death (Chace et al. 2001; Howat et al. 1984; Howat et al. 1985; Southall, Stebbens, and Shinebourne 1987; Arens et al. 1993). Laboratory methods that have been used in the study of IEM in children with sudden death include amino acid analysis by liquid chromatography or capillary electrophoresis, organic acid analysis by gas chromatography-mass spectrometry (GCMS) and functional studies on fatty acid oxidation and enzyme activity carried out in lymphocytes or fibroblasts. However, these methods are laborious and time consuming. Currently, it has become possible to investigate metabolites with only a small amount of blood and urine samples to make a biochemical diagnosis by development of mass spectrometric tools (Millington et al. 1990). Tandem mass spectrometry (TMS) in laboratory medicine has progressed from biomedical research application to routine clinical diagnostics application, including expanded newborn screening. TMS has revolutionized the investigation of IEM in neonatal death. TMS has the advantage of simultaneous multiple metabolites analyses which allows the detection of a wide range of common IEM disorder in a single analytical run. Using TMS, Chace et al. (2001) found 0.9% of highly suggestive cases of neonatal death due to IEM (Chace et al. 2001). Yet, the usage of high-risk reference ranges for IEM diagnosis in post-mortem samples is challenging. This is because TMS profiles in post-mortem blood specimens are characterised by non-specific, generalized large increases of most amino acids, free carnitine and short-chain acylcarnitines; C2, C3, C4, and C6 as well as hydroxybutyryl- (C4OH) carnitines (Chace et al. 2001). Hence, a specific post-mortem reference ranges is needed to screen for common IEM in post-mortem samples. In a retrospective study of 30 cases among Japanese infant presented with sudden unexpected death, two positive cases of long chain fatty acid oxidation defects were detected using the post-mortem blood acylcarnitine analysis and histological examination of the liver (Yamamoto et al. 2015). In view of this finding, there could be a high possibility of IEM amongst children with unexplained death in Malaysia. Furthermore, our pilot study on expanded newborn screening on IEM in Malaysia had showed a detection rate of 1 in 2916 (Yunus et al. 2016). Metabolic autopsy using the next generation whole exome sequencing (WES) is increasingly being used for post-mortem genomic study. This technique allows examination of larger number of genes and exons at a lower cost and time (Yamamoto et al. 2015). Unfortunately, WES is not widely available in diagnostic facilities, especially those in the less developed countries. This study was conducted to investigate common IEM as the cause of

sudden unexplained deaths in Malaysian children less than 5 years old using TMS as the screening platform. We proposed screening by TMS using appropriate post-mortem reference ranges for detection of IEM within this patient's cohort.

MATERIALS AND METHODS

Study design

Cross-sectional study from December 2012 to December 2014 involving five government hospitals in Malaysia; the Forensic departments in Hospital Kuala Lumpur, Hospital Sultan Ismail, Hospital Sultanah Aminah, Hospital Sultanah Nur Zahirah, Hospital Sultan Haji Ahmad Shah and Institute for Medical Research Kuala Lumpur. Ethical approval was obtained from the Medical Research and Ethics Committee Malaysia (MREC), Ministry of Health Malaysia and the study complied with Declaration of Helsinki.

Subjects and samples

Post-mortem samples were obtained from children aged 5 years old or below who died without a firm clinical diagnosis. Abandoned, decomposed bodies and stillbirths were excluded. 4 mL of whole blood and 2 mL of urine were requested for metabolic screening. Dried blood spots (DBS) were produced by spotting 50 µL of whole blood onto each circle of the Whatmann 903 filter paper and left to dry for 4 hours at room temperature. From the total 4 mL whole blood samples, 2 mL was aliquoted into each EDTA and heparin tube, respectively. The blood in the heparin tube was centrifuged at 3000 rpm for 5 min to obtain plasma for amino acid analysis. Urine was collected and sent for metabolic screening, whenever possible.

Laboratory analysis

Post-mortem blood analysis by TMS

The DBS samples were analysed for amino acids and acylcarnitines by electrospray TMS as the first line laboratory investigation. The methods by Millington et al. (1990) and Chace et al. (2001) were adopted with some modifications. 3 mm DBS was extracted using methanol containing the isotopic labeled amino acids and acylcarnitines standard. The samples were derivatised with butanolic chloride before injected onto the electrospray ionization outlet of the Micro mass TMS. The results were analysed by Microsoft Excel and SPSS version 21. All statistical analysis was considered significant when $p<.05$. High risk reference ranges were derived from 20,400 filter papers from symptomatic patients less than seven years old. Post-mortem reference ranges were developed from DBS of 122 post-mortem cases in children less than five years old.

Confirmatory biochemical tests

Confirmatory biochemical tests of plasma amino acid, urine organic and orotic acid were conducted. Plasma for amino acids was analysed using cation-exchange chromatography by a dedicated amino acid analyser. Urine orotic acid was analysed using a reverse phase high performance liquid chromatography (HPLC) system. Urine for organic acid analysis was subjected to organic solvent extraction and derivatisation prior to injection into the GCMS. In order to ensure the validity and reliability, quality control materials were also included for each analytical test.

Molecular analysis

Molecular analysis was conducted for samples that exceeded the cut-off during the biochemical screening whenever there is adequate DNA. Genomic DNA was extracted either from EDTA-blood sample or 3 mm DBS using standard protocol. A touchdown polymerase chain reaction (PCR) was carried out to amplify all the exons concurrently, occupying specific forward and reverse primers flanking the target genes (OTC, HADHA and HADHB genes). The forward primers were tailed with M13 FAM-labelled for ease of the downstream steps. After purification of PCR, cycle sequencing and purified, samples were then subjected to DNA sequencing which was performed in a fluorescent Genetic Analyzer ABI 3500 (Applied Bio Systems). Raw data was analysed for mutation using SeqScape software version 3.0 (Applied Biosystems). Any detected mutation was then compared with the Human Gene Mutation Database, HGMD (Basic) to determine whether the mutation has been previously reported. Further bioinformatics analysis was also conducted using MutationTaster free software (www.mutationtaster.org) to predict the alteration as polymorphism or disease causing (Schwarz et al. 2014).

RESULTS

Biochemical analyzed

DBS (n=138), plasma (n=59), whole blood (n=59) and urine sample (n=4) were analysed. Amino acids and acylcarnitines in the post-mortem samples showed

marked increased concentration compared to the high risk. Table 1 illustrates different cut-offs for post-mortem patients and sick alive patients (high risk). Eight DBS samples exceeded the cut-off limit by TMS screening test. There were two confirmed positive cases, four highly possible cases for fatty acid oxidation disorders and two highly possible cases for organic acid disorders (Table 2). Case 1 had elevation of glutamic acid at 530 µmol/L (<438 µmol/L) by TMS. Urine organic acid detected large peak of orotic acid and urine analysis by reverse phase HPLC showed marked elevation of orotic acid at 268 mmol/mol creatinine; (<5.3 mmol/mol creatinine) and plasma amino acid by cation exchange chromatography showed marked elevation of glutamine at 2762 µmol/L (<561 µmol/L), alanine at 2544 µmol/L (<546 µmol/L) and moderate elevation of glycine at 2544 µmol/L (<465 µmol/L). Citrulline was however within the reference range at 11 µmol/L (2-30 µmol/L) and arginine were below the low cut-off at 1 µmol/L (24-99 µmol/L). The biochemical profile is suggestive of Ornithine transcarbamylase (OTC) deficiency. Case 2 had normal glutamic acid at 293 µmol/L (<438 µmol/L) with mild elevation of alanine at 693 µmol/L (<488 µmol/L), with moderate elevation of C16OH acylcarnitines at 0.67 µmol/L and also, with raised C16OH/C16 ratio at 0.39 (p<.1). The acylcarnitine profile was highly presumptive positive for long chain 3-hydroxyacyl CoA dehydrogenase deficiency or mitochondrial trifunctional protein deficiency. Urine sample was not available for case 2.

In this study, we had found a positive correlation between C0, C2, C4, C4OH, C5OH, C6, C16OH, C18, total acylcarnitine, total carnitine, C4:C2, C4:C3, glycine, alanine, leucine/isoleucine, methionine, phenylalanine, tyrosine, glutamic acid, arginine with the sampling interval. Whereas, a negative correlation was found for C12:1, acylcarnitine:free carnitine, C8:C6, C3:C2, C5:C4, C5DC:C4, and C14:1:C4, C5DC:C5OH and

Table 1. Post-mortem vs high risk blood amino acids and acylcarnitines reference range by TMS

	Post-mortem				High risk patient		
	Median	**RR**	**Unit**		**Median**	**RR**	**Unit**
FC	82	17-382	µmol/L	FC	28.6	8.1-84.6	µmol/L
TC	150	33-470	µmol/L	TC	51.3	18- 146.3	µmol/L
AC/FC	0.7	0.19-1.33		AC/FC	0.78	0.39-1.56	
SCI	0.33	0.12-0.49		SCI	0.33	0.11-0.54	
MCI	0.01	0-0.03		MCI	0.01	0-0.03	
LCI	0.03	0.01-0.09		LCI	0.06	0.03-0.15	
C2	38	9-138	µmol/L	C2	14.6	3.1- 52.98	µmol/L
C3	3.85	0.66-9.3	µmol/L	C3	1.21	0.21-4.98	µmol/L
C4	2.2	0.4-16.0	µmol/L	C4	0.25	0.07-1.1	µmol/L
C4OH	1.23	0.1-7.0	µmol/L	C4OH	0.12	0.02-0.64	µmol/L
C5	0.58	0.13-3.2	µmol/L	C5	0.14	0.04-0.48	µmol/L
C5OH	0.32	0.08-1.38	µmol/L	C5OH	0.16	0.04-0.47	µmol/L
C5:1	0.1	0.02-0.72	µmol/L	C5:1	0.06	0.01-0.2	µmol/L
C5DC	0.14	0.05-0.41	µmol/L	C5DC	0.06	0.01-0.2	µmol/L
C8	0.3	0.08-0.95	µmol/L	C8	0.11	0.02-0.4	µmol/L
C10	0.26	0.05-1.29	µmol/L	C10	0.12	0.02-0.64	µmol/L
C10:1	0.14	0.02-0.54	µmol/L	C10:1	0.1	0.01-0.34	µmol/L
C12	0.3	0.04-1.8	µmol/L	C12	0.12	0.02-0.47	µmol/L
C12:1	0.13	0.02-0.52	µmol/L	C12:1	0.11	0.02-0.40	µmol/L
C14	0.33	0.09-1.32	µmol/L	C14	0.2	0.04-0.73	µmol/L
C14:1	0.2	0.04-0.76	µmol/L	C14:1	0.11	0.02-0.46	µmol/L

C14:2	0.1	0.03-0.47	µmol/L	C14:2	0.06	0.01-0.24	µmol/L
C16	1.56	0.33-6.22	µmol/L	C16	1.32	0.30-5.40	µmol/L
C16OH	0.1	0.01-0.44	µmol/L	C16OH	0.04	0.01-0.19	µmol/L
C16:1	0.23	0.07-0.93	µmol/L	C16:1	0.12	0.02-0.53	µmol/L
C18	0.81	0.15-2.44	µmol/L	C18	0.51	0.12-1.53	µmol/L
C18:1	1.03	0.29-3.09	µmol/L	C18:1	0.93	0.21-2.68	µmol/L
C18:2	0.29	0.08-1.1	µmol/L	C18:2	0.28	0.05-0.92	µmol/L
C3/C2	0.08	0.02-0.28		C3/C2	0.08	0.02-0.25	
C3/C16	2.52	0.36-12.16		C3/C16	0.89	0.17-4.33	
C5DC/C4	0.06	0.01-0.47		C5DC/C4	0.23	0.03-1.1	
C5OH/C3	0.09	0.02-0.67		C5OH/C3	0.13	0.02-0.73	
C5OH/C8	1	0.29-3.33		C5OH/C8	1.43	0.26-8.81	
C5OH/C0	0	0-0.01		C5OH/C0	0.01	0-0.02	
C8/C10	1.2	0.35-3.54		C8/C10	0.91	0.17-5.0	
C8/C6	0.55	0.19-2.82		C8/C6	1.23	0.21-6.0	
C8/C2	0.01	0-0.04		C8/C2	0.01	0-0.04	
C10:1/C10	0.57	0.09-2.0		C10:1/C10	0.83	0.09-4.73	
C14:1/C12:1	1.5	0.23-8.45		C14:1/C12:1	0.96	0.29-3.5	
C14:1/C16	0.14	0.04-0.51		C14:1/C16	0.08	0.03-0.25	
C16/C14:1	7.31	1.96-22.74		C16/C14:1	13	2.18-65	
C16OH/C16	0.07	0.01-0.44		C16OH/C16	0.03	0-0.22	
C16+C18:1/C2	0.07	0.02-0.19		C16+C18:1/C2	0.16	0.07-0.36	
C0/C16+C18	36.4	10-172		C0/C16+C18	15.5	4.08-52.77	
Alanine	1039	316-3636	µmol/L	Alanine	233	67-917	µmol/L
Glycine	618	57-1529	µmol/L	Glycine	219	76-725	µmol/L
Valine	283	104-762	µmol/L	Valine	169	69-348	µmol/L
Leucine	322	73-1301	µmol/L	Leucine	102	29-266	µmol/L
Methionine	68	17-251	µmol/L	Methionine	20	9.2-42	µmol/L
Phenylalanine	147	53-531	µmol/L	Phenylalanine	48.5	18-180	µmol/L
Tyrosine	109	12-435	µmol/L	Tyrosine	48.9	10-182	µmol/L
Glutamic acid	537	112-1806	µmol/L	Glutamic acid	203	68-557	µmol/L
Citrulline	14	13-73	µmol/L	Citrulline	12	3.3-35	µmol/L
Arginine	6	1.1-60	µmol/L	Arginine	3	0.53-21	µmol/ L

abbreviation:
FC=free carnitine
TC=total carnitine
SCI=short chain index
MCI=medium chain index
LCI=long chain index

Table 2. Clinical features and biochemical findings of positive and highly presumptive positive patients

Patient	Age	Sex	Consanguinity	Clinical features	Biochemical Findings	Diagnosis
1	3 days	M	No	Three days old male baby brought in dead to the emergency department.	Elevated glutamic acid & glutamine, raised orotic acid	OTC deficiency
2	5 days	F	No	IUGR, respiratory difficulty, hypoglycemia and feeding intolerance. There is history of neonatal death from maternal side. Patient is the first child in the family	Raised C16OH, C161, C16OH:C16 and total OH.	MTP deficiency
3	2 mths	M	No	Ex-premature baby. He had cough and fever with vomiting and diarrhea for three days and were found to be lethargic. Parents did not seek treatment until patient became comatose. Unsuccessful resuscitation	Raised C6, C8, C10, C5DC, C12 and low C101:C10.	MADD
4	10 mths	M	No	Fever, vomiting and diarrhea. Had poor oral intake. Found unconscious by mother.	Raised C8, C10 and low C101:C10.	MADD
5	1 mth	M	No	Septicaemic-like illness, failure to thrive, lethargic, cyanosed with respiratory difficulties.	Raised C8, C10, C5DC and C14. Low C101:C10	MADD
6	8 mths	M	No	Ex- premature baby was brought in dead to the emergency department. History of fever, cough with respiratory difficulties and lethargy.	Raised C8, C10, C10:1 and low C101:C10.	MADD
7	1 mth	F	No	History of frequent vomiting. Patient was already unresponsive upon arrival in the emergency department.	Raised C3, C3:C2 and C3:16	PPA/MMA
8	1 mth	M	No	Brought in dead to the emergency department.	Raised C3, C3:C2 and C3:C16	PPA/MMA

Table 3. Comparison of post-mortem acylcarnitine reference range in two studies

	Median (IQR)	
	Our study	**Pryce et al. (2011)**
AFR	0.7 (0.51-0.84)	0.59 (0.47-0.75)
SCI	0.33 (0.26-0.38)	0.36 (0.31-0.42)
MCI	0.01 (0.006-0.012)	0.0032(0.0026-0.0044)
LCI	0.03 (0.017-0.042)	0.0032 (0.0023-0.0055)
C0	87.3 (56.5-163)	169 (114.5-221)
C2	38.65 (28.6-56)	90.8 (65.25-120.7)
C3	3.85 (2.3-5.5)	2.02 (1.35-3.175)
C4	2.18 (1.3-4.8)	4.34 (2.79-6.065)
C5	0.58 (0.37-0.8)	0.76 (0.545-1.065)
C6	0.62 (0.33-0.99)	0.66 (0.42-0.86)
C8	0.3 (0.19-0.46)	0.22 (0.16-0.32)
C14	0.34 (0.23-0.6)	0.09 (0.06-0.15)
C16	1.56 (0.975-2.25)	0.8 (0.5-1.2)

abbreviation:
AFR=acylcarnitine/free carnitine ratio
SCI=short chain index
MCI=medium chain index
LCI=long chain index
IQR=interquartile range

C5:C0. All analytes showed skewed distributions, thus a non-parametric analysis was performed. When the age was grouped into 4 groups; 0-12 months, 13-24 months, 25- 36 months and 37-52 months, there was no significant difference in the mean concentration of all analytes among the age groups as compared using Kruskal - Wallis test. There was no significant difference in the concentration of all analytes between genders (p>.1), as determined using Mann Whitney/Wilcoxon rank test. The median age for all samples was 2 months old.

Mutation study

We found a hemizygous mutation in Case 1 at c.1A>T (p.Met1Leu) in exon 1 of OTC gene. This mutation caused a substitution of amino acid methionine to leucine in first codon (Figure 1). This mutation had been previously reported in HGMD by Yamaguchi et al. (2006) Screening for the parental samples was not done as the DNA samples were not available. In Case 2, the patient's DNA was inadequate to proceed for HADHA and HADHB gene analysis. Initially the molecular analysis showed one heterozygous mutation at c.1465A>G (p.Lys489Glu) in exon 14 of HADHA gene in patient's sibling. The subsequent analysis for HADHB gene showed a compound heterozygous mutation at c.182G>A (p.Arg61His) in exon 4 and at c.954de1G (p.Met318Ilefs*2) in exon 11 (Figure 2). Mutation c.954de1G is novel and had not been reported in the HGMD. Both mutations were inherited from the parents. Therefore, we presume that case 2 had the same mutations as the sibling.

DISCUSSION

The common practice in Malaysia in the investigations of unexplained deaths in children is first autopsy, followed by histopathology, microbiology and toxicology studies. Screening for IEM in children with unexplained death was not routinely done prior to 2012 partly due to unavailability of post-mortem reference ranges. Diagnosis of even common IEM is challenging in post-mortem patients. This is due to the generalised elevations of amino acids and the short chain acylcarnitines level when high risk or newborn screening cut-offs are used. In our laboratory practice, most cases will require screening by TMS before proceeding with confirmatory biochemical test.

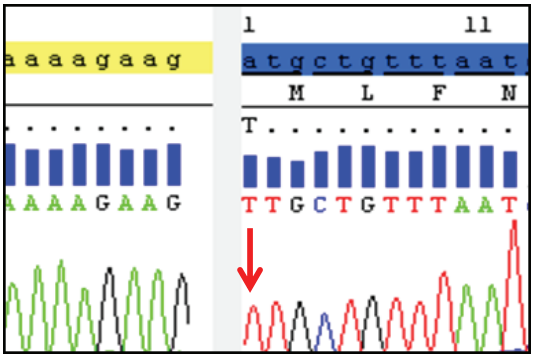

Figure 1. A hemizygous mutation at c.1A>T (p.Met1Leu) in exon 1 of OTC gene in Case 1.

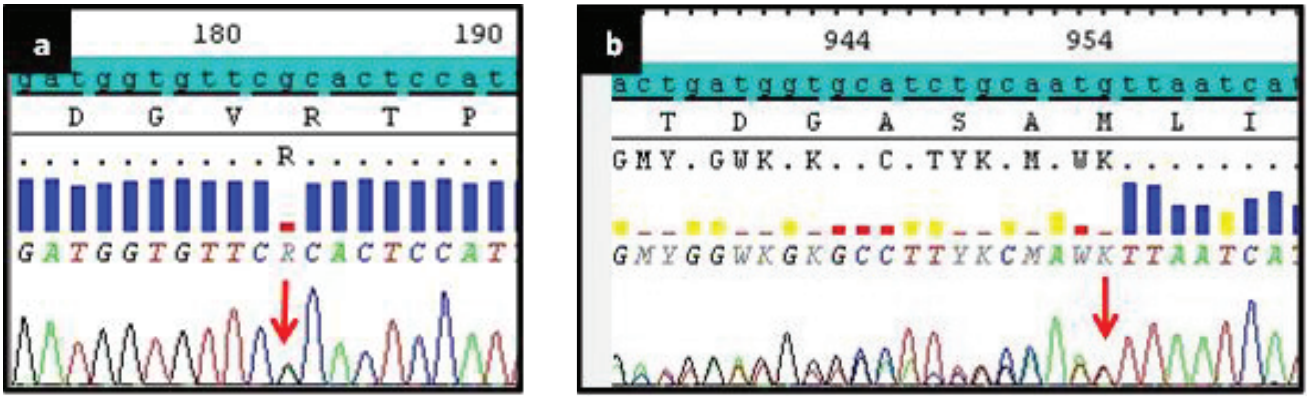

Figure 2: A compound heterozygous mutations in HADHB gene in Case 2. a) Mutation at c.182G>A (p.Arg61His) in exon 4. b) Mutation at c.954de1G (p.Met318Ilefs*2) in exon 11.

Thus, it is the foremost important task to get the correct cut-offs of the screening method for diagnosis of IEM in post-mortem patients. Despite of the advantages, there are also several limitations in our study. TMS can only screen amino acid disorders, organic acidurias, urea cycle defects and mitochondrial fatty-acid. However, most studies on the occurrence of IEM in children with sudden death had shown mitochondrial fatty acid oxidation and organic acid disorders to be the major cause of death. Hence, the approach of using TMS to screen for common IEM is fairly reasonable. Furthermore, the IEM cases that were detected by TMS from our pilot study was Maple syrup urine disease, methylmalonic aciduria, ethylmalonic aciduria, argininosuccinate lyase deficiency and isovaleric aciduria (Yunus et al. 2016). Nevertheless, there is a possibility for other group of IEM disorders such as the mitochondrial disorders in particular the respiratory chain diseases are not being diagnosed in the patient's cohort. Direct bladder puncture is commonly applied for urine sampling in post-mortem patients. Unfortunately, urine specimens may not be always available in view of involuntary emptying of bladder during the agony period (of death). Due to the unavailability of urine samples in most deceased subjects, we have validated method for plasma organic acid analysis for post-mortem subjects. One of the main challenges that we experienced is the lack of obtaining complete set of samples since the blood samples from post-mortem subjects require several biochemical investigations including toxicology. In our study, the concentration of the amino acids and most acylcarnitines by TMS correlates well with the sampling time. The sampling interval ranges from 20 minutes to 28 hours in all samples, but the median sampling interval was five hours and the mean was 9 hours. Our result findings are quite comparable to Chace et al. (2001) and Pryce et al. (2011).

The highly possible cases in this study were not confirmed due to either inadequate DNA materials or absence of urine samples. Diagnosis of IEM in plasma amino acid, organic acid and fatty acid oxidation disorder without urine sample and adequate patient clinical information is absolutely challenging as urine sample for organic acid analysis is a pre-requisite in the diagnosis of organic acid and fatty acid oxidation disorders considering the similar acylcarnitine abnormalities in the TMS will appear for some types of organic acid and fatty acid oxidation disorders. The samples are considered

precious since the patient is dead. The significance of bladder puncture for urine sampling at autopsy cannot be over-emphasized. Although direct bladder puncture is commonly practice in Malaysia for urine sampling in the deceased, unfortunately urine specimens may not be always available for post-mortem analysis due to involuntary emptying of bladder during the agony period of death. Screening for IEM by TMS using the DBS has the advantage of being stable at transportation, requires low volume of blood and long storage stability. It is also a source of DNA materials, although DNA extracted from DBS is often very low in yield and not sufficient to analyse genes with bigger exons. The next generation sequencing (NGS) is currently the most recent available method especially in the developed countries to screen for multiple genetic disorders using relatively low volume of samples. The NGS targeted panel can screen for multiple genetic disorders at a lower cost and complexity of variant interpretation, however, it still requires highly skilled personnel for interpretation. Nevertheless, the death in this cohort of patients could have been prevented by expanded screening by TMS which aim to detect diseases before it manifests, thus significantly reducing morbidity and mortality. Mass spectrometry has emerged as a niche technology in routine laboratory medicine and it is very likely to remain the niche technology in the future (Seger and Salzmann 2020). In the last two decades, reagents for expanded screening by TMS have become commercially available and are being used by many clinical laboratories (Lehotay et al. 2011). However, as a screening test TMS is not diagnostic and should not be assumed to confirm the absence or presence of the disease. Thus, whole blood for DNA, plasma and urine should be made available in the investigation of IEM in post-mortem samples for confirmatory of disease and accurate post-mortem diagnosis of IEM to be established. A small punched skin biopsy measuring 4 mm in diameter is useful for investigations of mitochondrial and respiratory chain diseases and can serve as a good source of DNA material.

CONCLUSION

Appropriate cut-offs for post-mortem samples allow detection of common IEM in children with sudden death. In combination with molecular genetics, accurate post-mortem diagnosis for IEM can be made. Screening by TMS for common IEM should be carried out for all children presented with sudden death.

ETHICAL DECLARATIONS

Ethical approval was obtained from the Medical Research and Ethics Committee Malaysia (MREC) and next of kin were consented to participate in the research project.

CONFLICT OF INTERESTS

The authors declare no conflict of interest involve in this study.

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