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Tandem Mass Spectrometry in Patients with Drug Resistant Epilepsy

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ABSTRACT

Background: Inherited metabolic abnormality was a common influential factor in the pathogenesis of intractable epilepsy. Screening of inborn metabolic abnormality in children with intractable epilepsy should be conducted as early as possible, to achieve early treatment and improve their prognosis.

Methods: Descriptive study was conducted in Outpatient Neurology Clinic –Ain Shams University Pediatric Hospital. It included 30 (12 male and 18 female) patients with intractable epilepsy during the period from February 2017 to December 2017. All patients presented with drug resistant epilepsy. All included patients were subjected to full history talking , clinical examination and were investigated by serum lactate, serum ammonia, arterial blood gases, Extended Metabolic Screen using tandem mass spectrometry, urinary organic acids, fundus examination, EEG and neuroimaging.

Results: Abnormal urinary organic acid analysis was present in 5 patients as follows: 3-hydroxyglutaric acid in one patient, increase lactic acid in three patients and 2-oxoglutaric in one patient. Plasma amino acid analysis results were alanine elevation in 4 patients, elevated C5-DC in one patient, abnormal co-carnitine in three patients, 2 of them had low concentration and one had high concentration, elevated glycine in two patients and phenylalanine elevation in only one .**Conclusion:** Inherited metabolic abnormality was a common influential factor in the pathogenesis of intractable epilepsy.

Keywords: Tandem mass spectrometry, intractable epilepsy.

INTRODUCTION

Drug resistant epilepsy may be defined as failure of adequate trials of two tolerated and appropriately chosen and used antiepileptic drug (AED) schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom. It is important to note that no seizure frequency requirement is necessary to meet the definition. Thus, an individual with one seizure per year can be regarded as treatment resistant [1].

Inherited metabolic abnormality was a common influential factor in the pathogenesis of intractable epilepsy (IE), especially in infantile spasms. Screening of inborn metabolic abnormality in children with IE should be conducted as early as possible, to achieve early treatment and improve their prognosis [2].

Inborn error of metabolisms are a collection of rare genetic diseases that generally result from a deficiency of an intracellular component (e.g., an enzyme or transporter) of a metabolic pathway, resulting in an accumulation of a substrate or intermediate in a pathway and/or reduced ability to synthesize essential compounds. Often the central nervous system (CNS) is affected, leading to neurological disease [3].

Although these disorders are individually rare, collectively they account for a significant portion of childhood disability and deaths. Most of the disorders are inherited as autosomal recessive whereas autosomal dominant and X-linked disorders are also present. The clinical signs and symptoms arise from the accumulation of the toxic substrate, deficiency of the product, or both. Depending on the residual activity of the deficient enzyme, the initiation of the clinical picture may vary starting from the newborn period up until adulthood [9].

The following clues should raise the suspicion of an inherited metabolic disorder: the marriage is consanguineous; there is a history of recurrent abortion; there is a history of unexplained neonatal death in siblings especially associated with acidosis, coma and convulsions, picture like encephalopathy, and a sibling has been diagnosed as suffering from an inborn error of metabolism (IEM) [8]. In certain developed and developing countries, neonatal screen for metabolic disorders allows diagnosis and treatment in the pre-clinical phase, so that the adverse consequences of such disorders can be prevented. Metabolic disorders for which newborn screening are conducted include phenylketonuria (PKU), galactosaemia, maple syrup

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urine disease (MSUD), homocystinuria, and biotinidase deficiency [4].

PARTICIPANTS AND METHODS

Descriptive study was conducted in Outpatient Neurology Clinic –Ain Shams University Pediatric Hospital. It included 30 (12 male and 18 female) patients with intractable epilepsy during the period from February 2017 to December 2017. Inclusion criteria included intractable epilepsy-patients ageing from 1-5 years. Exclusion criteria included head injury, tumor, congenital abnormalities, drugs toxicity and CNS infections.

Ethical considerations:

An informed consent was obtained from parents or care givers of all subjects prior to enrollment. **The study was approved by the Ethics Board of Ain Shams University.**

Study measurements:

The patients were subjected to detailed medical history taking and physical examination with special emphasis on anthropometric measure, occipitofrontal circumference hepatosplenomegaly, dysmorphic features, signs of meningeal irritation, localization signs, signs of neurocutaneous syndrome and skin lesion.

All patients were investigated by CBC, serum lactate, liver enzymes, serum ammonia, arterial blood gases, Extended Metabolic Screen using tandem mass spectrometry, urinary organic acids, fundus examination, EEG, and neuroimaging.

Statistical methods

Statistical analysis was done using manual methods to calculate percentage of the obtained data of the patients.

RESULTS

Clinical data of intractable epilepsy patients showed 5 positive consanguinity, 4 positive family history, 22 delayed motor development, 24 delayed mental development. One patient had macrocephaly and one microcephaly. One had abnormal movement and 10 delayed speech.

Other manifestations included failure to thrive in 10, over weight in one patient, recurrent chest infection in 5, recurrent GE in 12, short stature in 8 patients, fair hair in one patient, and hepatomegaly in one patient.

Laboratory investigations showed that 7 patients had elevation of serum ammonia, 4 patients had metabolic acidosis, 8 patients had elevation of serum lactate, while serum creatinine, serum urea were within normal. Abnormal urinary organic acid analysis was present in 5 patients as the follows: 3-hydroxyglutaric acid in one patient, increase lactic acid in three patients and 2-oxoglutaric in one patient. **(Table 1).**

Plasma amino acid analysis results were alanine elevation in 4 patients, elevated C5-DC in one patient, abnormal co-carnitine in three patients, 2 of them had low concentration and one had high concentration, elevated glycine in two patients and phenylalanine elevation only one **(Table 1)**.

Neuroimaging brain for 30 patients showed atrophic changes in 9 patients, basal ganglia calcification in 2 patients, and white matter abnormalities in 4 patients**(Table 2)**.

EEG showed 8 patients with multifocal epilepsy, 13 patients with focal activity and 9 with generalized activity**(Table 3)**.

Table (1): Comparisons of the screening results of urine and blood on possible IEM in studied patients, according to ***Liu et al. (2016).***

Blood screening	Urine screening	Possible clinical diagnosis
High C5-DC	3-hydroxyglutric acid	Glutaric academia type 1
elevated anlanine and co carnitine	Elevated lactic acid	Possible mitochondrial Cytopathy
Elevated ornithine and phenyl alanine	Normal	Possible phenylketonuria
"Elevated alanine and Glycine and Low co-carnitine"	Elevated lactic acid	Possible mitochondrial Cytopathy
Elevated alanine	Elevated lactic acid	Possible mitochondrial Cytopathy
Low level of co, c2, c3, elevated alanine and glycine	Elevated lactic, 2-oxoglutaric acid	Possible mitochondrial Cytopathy

Table (2): CT brain Findings in 30 patients:

	Number	**Percentage**
CT abnormalities	11	37%
Cerebral atrophy	9	17%
Basal ganglia abnormalities	2	7%
No abnormality	19	63%

Table (3): EEG Findings in 30 patients:

	Number	Percentage
Multifocal activity	8	27%
Focal activity	13	43%
Generalized activity	9	30%

DISCUSSION

Diagnosis of IEM was not conclusive in 6 patients, however since they have very suggestive symptoms and signs, they need further investigations. Incidence of various classes of disorders in Egypt (phenylketonuria [1:5,000], methylmalonic acidemia, and isovaleric acidemia [1:12,500]), maple syrup urine disease, propionic acidemia, b-ketothiolase deficiency, and primary carnitine deficiency [1:25,000] giving a total birth prevalence of 1:1944 live births [3].

In our study, the first manifestations of these patients were recorded at age of <1 year in 67% of all patients and from 1 to 5 years in 33% of patients this agrees with **Chi [1]and Lee *et al.*** [5] Certain IEM are more prevalent in particular ethnic or religious groups and negative family history does not rule out an IEMs because most carriers have no clinical manifestations of disease [1].

In our study neurologic manifestation were the commonest observed manifestation. Seizures were observed in (100%), delayed motor and mental development in 100% of patients, failure to thrive in 50%, 17 % skin changes, elevated ammonia and lactate 83%, metabolic acidosis, abnormal EEG finding in 100% and neuroimaging abnormalities in (83%). These symptoms caused mostly by defective enzymes or transporters in metabolic pathways. Such defects lead to malfunctioning metabolism and accumulation of toxic intermediate metabolites [6]. This agrees with **Liu *et al.*** [7] study who applied metabolic screening to detect the blood and urinary metabolic components of 56 IE children, and 25 cases were confirmed with 12 types of abnormalities, (76%) confirmed with backward or regressive intelligent movement; cases (55.5%) with skull imaging abnormalities, (24.2%) with blood biochemistry and blood gas analysis abnormalities, including the increase of blood ammonia, and blood lactic acid and (18.5%) with skin change.

In current study, four patients had abnormal elevated amino acids with age 1 to 5 years(67% of positive case), this is in contrast with the study by ***Liu et al.*** [7] where 8% of his cases, respectively, had possible mitochondrial dysfunction.

In our study, one patient of 6 positive cases had highly suggestive symptoms of organic academia type 1 (16%), this is in agreement with ***Lui et al.*** [7] 1 of the 25 cases of children with intractable epilepsy had abnormalities in urine or blood screening, one diagnosed as organic academia type 1who had delayed motor and mental, metabolic acidosis and neuroimaging abnormalities.

In our study one patient possible Phenylketonuria (16%) of positive cases and (3%) of all patients, this in contrast with ***Hassan et al.*** [3] who diagnosed 235 with 20 different IEMs, PKU was the most common single IEM detected in high-risk cases (116/235 (49.3%) .And disagreed with ***Lui et al.*** [7] who diagnosed 25 of 56 intractable epileptic patients IEM, 3of 25 (12%) were PKU.

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