ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Simple Urine Metabolic Screning In Children With Refractory Epilepsy In Zagazig University Children's.

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**ABSTRACT**

Epilepsy is one of the most common serious neurological conditions and about 20%to 30% of individuals with epilepsy have repeated seizure attacks that develop refractory epilepsy .The aim of the present study is to examine the inherited metabolic abnormalities in children with refractory epilepsy to provide early etiological and symptomatic treatment. Patients may be exposed to refractory epilepsy if the application of two types of appropriate and tolerable antiepileptic drugs failed to completely prevent epileptic seizure following adequate duration of treatment and adequate doses of drugs, Refractory epilepsy always has been a serious issue in neurology particularly in infantile period when the brain of children develops rapidly. An expanding number of children with refractory epilepsy are mainly focused on genetic metabolic disorders .Since most inherited metabolic disorders are deficient in specific manifestations; they are not entirely obvious or missed in clinical analysis. This study was applied to forty eight patients with refractory epilepsy by application of urine metabolic screen by using urine test strips, Benedict test, ferric chloride test, Nitrosonaphthol test, Cyanide-nitroprusside test,2. 4 dinitrophenyl hydrazine test. In our study regarding urine screening tests 42% of our cases had presented positive results in at least one of the urine screening tests. This denotes the importance of urine screening tests for early detection of metabolic diseases among cases of refractory epilepsy. Compared with the positive rate of the urine metabolic screening tests in high risk infants suggest probability of the presence of a metabolic disease, which will need further investigations.

**Keywords:** refractory epilepsy, metabolism, Screening

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ISSN: 0975-8585

## INTRODUCTION

Epilepsy constitutes one of the common children's neurological disease [1]. Refractory epilepsy May be defined as failure of total or satisfactory control of seizures in response to anti-epileptic drugs. For routine use, failure of seizure-control despite trial of three appropriate anti-epileptic drugs (AED) regimens at maximum tolerated doses should be considered as drug- refractoriness and is very liable to show disappointment with subsequent AED failures.

Pseudo or apparent refractoriness is an important concern in refractory epilepsy and it may be confused in diagnosis ,so It should be excluded before diagnosis of refractory epilepsy [2]**.** The factors to be considered in eplipsy control are , appropriateness of the drug for the epilepsy syndrome, compliance, seizure mimickers, adequate dosing , trial and drug interactions .The pathogenesis of refractory epilepsy is complicated, and still need to be clarified and be well explained . The development of molecular genetics and biochemistry have led us to that refractory epilepsy is closely associated with inherited metabolism [3], [4].

The expanding number of children with refractory epilepsy are mostly centered around hereditary metabolic disorders [5].Inherited metabolic disorders are a series of metabolic defects of different clinical manifestation, including organic acids, amino acids, fatty acids and metabolic disease, which are incurred by disorder of biochemical metabolism and the accumulation of toxic products and the accumulation or insufficiency of metabolites resulting from the structural defects or functional disorders of protein arising from gene mutation[6], [7] **.**

The symptoms that are occurred mainly depend on the particular metabolic pathway that is affected [8]**.**Clinical features of metabolic disorders are non-specific and none of these features alone may point towards a metabolic disorder, so the ongoing advances in the finding and treatment of inherited metabolic issue have enhanced the determinations for a large number of these condition[9]

**Aim of study :** This study is aimed to examine metabolic abnormalities in children with refractory epilepsy to provide early etiological and symptomatic treatment to provide the best opportunity to prevent adverse psychological and social consequences of recurrent seizures, irreversible disability and premature death.

## PATIENT AND METHODS:

### A) Site of the study:

T

his study will be carried out at General and metabolic Unit of Zagazig University Children's Hospital over a six months period.

### B) Sample size:

The sample size will be about 48. Assuming that the expected number of cases with refractory epilepsy fulfilling the inclusion criteria admitted in Zagazig University Children's Hospital is about 8 per month and all of them will be included in the study as a comprehensive sample.

### C) Target population:

**Inclusion criteria:**

All patients conformed to the unified criteria of refractory epilepsy.

The unified criteria for the present study were : clinical diagnoses was exact ,the application of two types of appropriate and tolerable antiepileptic drugs failed to completely prevent epileptic seizure following adequate duration of treatment and adequate doses of drugs, or patients were clinical confirmed as refractory epilepsy syndrome.

**Exclusion criteria:**

- Perinatal cerebral injury
- Intra cranial mass
- Head trauma
- Poisoning

**Method**

This study includes 48 patients with refractory epilepsy, cross section study . Urine metabolic screening for all subjects: 25 ml of random sample of urine was collected from each patient in a sterile plastic container containing 0.5 ml of 6N HCl as preservative. Urine sample was screened for any physical variations in color, appearance, pH, specific gravity. using urine test strips ,Benedict test**,** ferric chloride test, Nitrosonaphthol test, Cyanide-nitroprusside test**,**2,4 dinitrophenyl hydrazine test.

**Statistical analysis**

Data were analyzed by Statistical Package of Social Science (SPSS), software version 24.0 **(SPSS Inc., 2016).**

**Continuous data** were presented as Mean±SD if normally distributed or Median (Range) if not normally distributed.

**Categorical data** were presented by the frequency and percentage. Normality was checked by Shapiro-Wilk test.

- **Chi-squared test of association**: is used to discover if there is a relationship between two categorical variables.
- **Fisher's Exact Test:** (alternative to Chi-squared test) when one or more expected cell frequencies less than five in a 2 x 2 crosstabulation.
- **Independent-samples t-test**: is used to determine if a difference exists between the means of two independent groups on a continuous dependent variable.
- **Mann-Whitney u test** (nonparametric alternative to independent-samples t-test)

**RESULTS**

Urine screening tests 42% of our cases had shown positive results of , Benedict test was positive in 17% of cases, 2,4 dinitrophenyl hydrazine test was positive in 4% of our patients ,Nitrosonaphthol test was positive in 6% of our patients, Ferric chloride test was positive in 13% of our patients and cyanide nitroprusside test was positive in 2% of our patients.(**table 1**).

**Table 1: Family and developmental history of the studied children**

| Variables | | n | % |
|---|---|---|---|
| **Consanguinity** | Negative | 22 | 46 |
| | Positive | 26 | 54 |
| **Unexplained illness or death** | Negative | 28 | 58 |
| | Positive | 20 | 42 |
| **Developmental delay** | Negative | 13 | 27 |

ISSN: 0975-8585

| | | | |
|---|---|---|---|
| | Positive | 35 | 73 |

Total number=48

**Table 2: Clinical characteristics of the studied children**

| Variables | | n | % |
|---|---|---|---|
| Dysmorphic facies | Negative | 17 | 35 |
| | Positive | 31 | 65 |
| Jaundice | Negative | 39 | 81 |
| | Positive | 9 | 19 |
| Seizure type | General | 28 | 58 |
| | Partial | 13 | 27 |
| | General+ Partial | 7 | 15 |
| Hypotonia | Negative | 29 | 60 |
| | Positive | 19 | 40 |
| Organomegaly | Negative | 35 | 73 |
| | Positive | 13 | 27 |
| Vomiting | Negative | 22 | 46 |
| | Positive | 26 | 54 |
| Episode of coma or encephalopathy | Negative | 23 | 48 |
| | Positive | 25 | 52 |

**Table 3: Data of urine screening tests**

| Variables | | n | % |
|---|---|---|---|
| Benedict test | Negative | 40 | 83 |
| | Positive | 8 | 17 |
| DNPH | Negative | 46 | 96 |
| | Positive | 2 | 4 |
| Nitrosonaphthol | Negative | 45 | 94 |
| | Positive | 3 | 6 |
| Cyanide nitroprusside | Negative | 47 | 98 |
| | Positive | 1 | 2 |
| Ferric chloride | Negative | 42 | 87 |
| | Positive | 6 | 13 |
| Toluidine blue | Negative | 48 | 100 |
| | Positive | 0 | 0 |

ISSN: 0975-8585

**Table 4: Patients' Family and developmental history & positive urine screen tests**

| Family and developmental history | Benedict test | DNPH | Nitrosonaphthol | Cyanide nitroprusside | Ferric chloride |
|---|---|---|---|---|---|
| **Consanguinity** | | | | | |
| n | 5/26 | 0/26 | 2/26 | 1/26 | 3/26 |
| % | 19% | 0% | 8% | 4% | 11.5% |
| **Unexplained illness or death** | | | | | |
| n | 3/20 | 2/20 | 1/20 | 1/20 | 5/20 |
| % | 15% | 10% | 5% | 5% | 25% |
| **Developmental delay** | | | | | |
| n | 5/35 | 2/35 | 3/35 | 1/35 | 4/35 |
| % | 14% | 6% | 9% | 3% | 11% |

**Table 5: Patients' clinical characteristics and positive urine screening tests**

| Clinical characteristics | Benedict test | DNPH | Nitrosonaphthol | Cyanide nitroprusside | Ferric chloride |
|---|---|---|---|---|---|
| **Dysmorphic facies** | | | | | |
| N | 4/31 | 2/31 | 3/31 | 1/31 | 5/31 |
| % | 13% | 7% | 10% | 3% | 16% |
| Jaundice, n | 2/9 | 0 | 0 | 0 | 1/9 |
| **Seizure type** | | | | | |
| **General,** | | | | | |
| N | 6/28 | 2/28 | 2/28 | 0/28 | 4/28 |
| % | 21% | 7% | 7% | 0% | 14% |
| Partial, n | 2/13 | 0/13 | 0/13 | 0/13 | 0/13 |
| General+ Partial, n | 0/7 | 0/7 | 1/7 | 1/7 | 2/7 |
| Hypotonia, n | 0/19 | 2/19 | 1/19 | 0/19 | 3/19 |
| Organomegly, n | 5/13 | 0/13 | 2/13 | 0/13 | 2/13 |

**Table 2** demonstrate the characteristics of children included in the study .about 65% have dysmorphic facies , 19% have jaundice ,15 % have general type and partial seizures and about 40 have hypotonia .

According to urine screening tests results , about 17% of total children have positive bendic test, DNPH was positive in about 4%, Nitrosonaphthol was positive in about 6% and Cyanide nitroprusside was positive in about 2% .as shown in (**table 3**).

We assess the family and developmental history of patients with positive screen tests and we found that about 19% of benedict test have consanguinity and about 4% of cyanide nitroprusside have consanguinity. About 25% of patients have positive ferric chloride had positive unexplained illness or death in family .while 14% of patients with benedict test positive have family developmental delay.as shown in (**table4**).

Also, we assess the characteristics of patients who have positive urine tests , we found that about 16% of ferric chloride patients have dysmorphic facies (the largest portion) while 3% of cyanide nitroprusside have dysmorphic facies (the smallest portion ). According to jaundice 1 from feeric group and 2 from benedict group observing to have jaundice, while others do not have .and according to seizures about 21% of benedict group have general siezures and about 7% in DNPH and Nitrosonaphthol develop general seizures while 14% in ferric and no one in nitroprusside group.in benedict group there is 5 patients have organnomegaly .as shown in (table 5)

**DISCUSSION**

The inherited errors of metabolism (IEM) are the consequences of genetic defects that lead to a metabolic suppress in a biochemical pathway which is critical to cellular function, targeting many organs including the brain. The underlying biochemical disturbance results in neuronal dysfunction and development of epileptogenesis through complex cellular mechanisms that may be specific to each disorder. Epilepsies associated with IEM are identical in certain featuressuch as , early age of presentation, co-morbid developmental delay/regression and resistance to conventional antiepileptic drugs [8]**.**The cross sectional study was carried out at the pediatrics department and clinical pathology department of zagazig university hospitals to study Inborn errors of metabolism in infants and children with refractory epilepsy for early intervention and proper treatment to be provided ,thereby effectively controlling patient seizures and improving their distant prognosis In our study developmental delay was the predominant symptom (73%),dysmorphic facies(65),consanguinity(54),vomiting(54%).episodes of encephalopathy(52), and hypotonia(40%)**""**[10]and This in agreement with a study conducted in Neurology Department, Cairo University Children Hospital by (Laila et al., 2009)[11] on Eight hundred patients attending the neuro-metabolic clinic at Cairo University Children Hospital (CUCH) screened for inborn errors of metabolism (IEM) by MS/MS. Developmental delay was a dominant symptom being present in (84.2%), vomiting in (63.2%), encephalopathy with episodes of encephalopathy (57.8%), seizures in (42%). IEM may be accompanied by developmental delay or regression, seizures, nystagmus, abnormal muscular tonus, or abnormal movements. Recurrent episodes of lethargy, ataxia, seizures, or strokes can show an underlying metabolic disorder such as ornithine transcarbamylase deficiency, organic acid disorders, maple syrup urine disease, homocystinuria, and mitochondrial disease. Patients with creatine synthesis defects may present with growth failure and seizures [12]. Vomiting is commonly episodic in metabolic disorders and leads frequently to failure to thrive (FTH). worsening of vomiting during current illnesses and lethargy with acidosis are highly suggestive of an underlying metabolic disease such as urea cycle defects, organic acid disorders, amino acid disorders, hereditary fructosemia, galactosemia, fatty acid oxidation defects, or mitochondrial disorders [13]. Regarding consanguinity in our case group, it was present in 54% of all cases. Of these cases 19 % had positive Benedict test, 8 % had positive Nitrosonaphthol test, 4 % had positive cyanide nitroprusside test and 11.5 % had positive ferric chloride test. These results are in agreement with [14]who found a significant incidence of consanguinity among metabolic diseases.

In this study 20 out of 48patients (42%) who were included in the study had siblings with unexplained illness or death. Of these cases 15 % had positive Benedict test, 10 % had positive DNPH test, 5 % had positive Nitrosonaphthol test, 5 % had positive Cyanide nitroprusside and 25 % had positive ferric chloride test.

These results are in agreement with [15]**.** In this study 9 out of 48 patients (19%) who were included in the study presented with jaundice in association with *refractory epilepsy.* 2 of these cases had positive Benedict's test and 1 had positive ferric chloride.

Inborn errors patients may present with one any of the characteristic features of liver disease. Conjugated or unconjugated hyperbilirubinemia can be caused by IEM. Patients with classical galactosemia usually present with vomiting, lethargy, hyperbilirubinemia, and liver failure in the first weeks of life. Reye-like acute encephalopathy with hepatocellular dysfunction is highly suggestive of fatty acid oxidation defects. Conjugated hyperbilirubinemia associated with some dysmorphic features and hearing loss can be caused by a defect in peroxisomal biogenesis. Neonatal cholestasis can also be caused by bile acid synthesis or transport

defects, congenital glycosylation defects, tyrosinemia type 1, or lysosomal storage disorders such as Niemann-Pick disease type C. The coagulopathy associated with metabolic liver disease is often severe but may not be clinically apparent. Therefore, screening for liver dysfunction, when there is suspicious of an underlying metabolic disorder, it should always include prothrombin time, partial thromboplastin time, and fibrinogen.The presence of hepatosplenomegaly with slowly progressive psychomotor retardation suggests a diagnosis of a lysosomal storage disease [16].

In this study 31 out of 48 patients (65%) who were included in the study presented with dysmorphic facies in association with *refractory epilepsy* . 13% Of these cases had positive Benedict test, 7 % had positive DNPH test, 10 % had positive Nitrosonaphthol test, 3 % had positive cyanide nitroprusside test and 16 % had positive ferric chloride test. Structural cerebral abnormalities and/or mild dysmorphic features can indicate energy metabolism defects such as mitochondrial disorders

In this study 19 out of 48 patients (40%) who were included in the study presented with hypotonia in association with Refractory epilepsy .2 of these cases had positive DNPH test, 1 had positive Nitrosonaphthol test, 0 had positive cyanide nitroprusside test, 0 had positive toluidine blue test and 3 had positive ferric chloride test. In this study 13 out of48 patients (27%) presented with organomegaly in association with Refractory epilepsy,5 of these cases had positive Benedict's test, 0% had positive DNPH test, 2 had positive Nitrosonaphthol test, 0 had positive cyanide nitroprusside test, 0% had positive toluidine blue test and 2 had positive ferric chloride test.

In our study 25 out of 48 patients (52%) presented with episodes of coma in association with Refractory epilepsy,20% of these cases had positive Benedict's test, 8% had positive DNPH test, 12% had positive Nitrosonaphthol test, 0 % had positive cyanide nitroprusside test, 0% had positive toluidine blue test and 24 % had positive ferric chloride test. Coma, organomegaly, seizures, developmental delay are significantly present in association with inborn errors of metabolism in many center studies .Although each individual disease is rare but the whole sum of these metabolic disorders are not rare. The rarity of these disorders, different modes of presentation and atypical forms should raise the necessity for screening for these disorders to be early picked up, early treatment and possibly early detection of these disorders in their siblings.there is no significant association between clinical data and urine metabolic screening test in the studied children due to study limitation, which are:

1- Relatively small sample size
2- It was a single-center study (ZagazigUniversity)
3- Diagnosis of metabolic diseases requires more specific diagnostic tests.

## CONCLUSION

IEMs are a rare cause of epilepsy but seizures and epilepsy are common in IEM, where the central nervous system carries the principal burden of disease. Many of these disorders are amenable to specific treatments and hence timely and appropriate diagnosis is essential to prevent irreversible brain damage. This requires knowledge about the clinical presentation, standardized vitamin trials, and availability of biochemical tests for diagnosis. IEMs that remain undiagnosed by clinical and biochemical testing, next-generation sequencing with gene panels, and whole exome sequencing may increase the diagnostic yield Compared with the positive rate of the urine metabolic screening tests in high risk infants suggest probability of the presence of a metabolic disease, which will need further specific investigations to determine the possible treatable cases. This is of fundamental importance for directing the awareness of general practitioners and pediatricians towards the metabolic disorder.

## REFERENCES

[1] N. Durmus, T. Kaya, S. Gültürk, T. Demir, M. Parlak, and A. Altun, “The effects of L type calcium channels on the electroencephalogram recordings in WAG/RIJ rat model of absence epilepsy,” *Eur. Rev. Med. Pharmacol. Sci.*, vol. 17, no. 9, pp. 1149–1154, May 2013.

[2] M. J. Brodie, “Road to refractory epilepsy: the Glasgow story,” *Epilepsia*, vol. 54 Suppl 2, pp. 5–8, May 2013.

[3] Patrick Kwan, "Definition of drug resistant epilepsy: Consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies - Kwan - 2010 - Epilepsia - Wiley Online Library." [Online]. Available: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1528-1167.2009.02397.x. [Accessed: 12-Jul-2018].

[4] A. T. Berg *et al.*, "How long does it take for epilepsy to become intractable? A prospective investigation," *Ann. Neurol.*, vol. 60, no. 1, pp. 73–79, Jul. 2006.

[5] G. Vitiello *et al.*, "Epilepsy in inherited metabolic disorders: a pediatric series," *Minerva Pediatr.*, vol. 64, no. 5, pp. 513–520, Oct. 2012.

[6] H. Kaspar *et al.*, "Urinary amino acid analysis: a comparison of iTRAQ-LC-MS/MS, GC-MS, and amino acid analyzer," *J. Chromatogr. B Analyt. Technol. Biomed. Life. Sci.*, vol. 877, no. 20–21, pp. 1838–1846, Jul. 2009.

[7] X. LIU, R. LI, S. CHEN, Y. SANG, and J. ZHAO, "Screening of inherited metabolic abnormalities in 56 children with intractable epilepsy," *Exp. Ther. Med.*, vol. 12, no. 1, pp. 135–140, Jul. 2016.

[8] A. N. Prasad, S. Levin, C. A. Rupar, and C. Prasad, "Menkes disease and infantile epilepsy," *Brain Dev.*, vol. 33, no. 10, pp. 866–876, Nov. 2011.

[9] D. H. Campos, "[Tandem mass spectrometry as screening for inborn errors of metabolism].," *Rev. Med. Chil.*, vol. 139, no. 10, pp. 1356–1364, Oct. 2011.

[10] M. Shevell *et al.*, "Practice parameter: evaluation of the child with global developmental delay: report of the Quality Standards Subcommittee of the American Academy of Neurology and The Practice Committee of the Child Neurology Society," *Neurology*, vol. 60, no. 3, pp. 367–380, Feb. 2003.

[11] L. Selim *et al.*, *Screening for Organic Acid Disorders among Egyptian Children with Clinically Suspected Neurometabolic Disorders*, vol. 4. 2009.

[12] S. Stockler, P. W. Schutz, and G. S. Salomons, "Cerebral creatine deficiency syndromes: clinical aspects, treatment and pathophysiology," *Subcell. Biochem.*, vol. 46, pp. 149–166, 2007.

[13] K. Gibson, J. L. Halliday, D. M. Kirby, J. Yaplito-Lee, D. R. Thorburn, and A. Boneh, "Mitochondrial oxidative phosphorylation disorders presenting in neonates: clinical manifestations and enzymatic and molecular diagnoses," *Pediatrics*, vol. 122, no. 5, pp. 1003–1008, Nov. 2008.

[14] M. M. Mokhtar, S. M. Kotb, and S. R. Ismail, "Autosomal recessive disorders among patients attending the genetics clinic in Alexandria," 1998.

[15] M. T. Greenberg, "Attachment and psychopathology in childhood," in *Handbook of attachment: Theory, research, and clinical applications*, New York, NY, US: Guilford Press, 1999, pp. 469–496.

[16] J. M. Saudubray, H. O. de Baulny, and C. Charpentier, "Clinical Approach to Inherited Metabolic Diseases," in *Inborn Metabolic Diseases*, Springer, Berlin, Heidelberg, 2000, pp. 3–41.