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Urine Metabolic Screening in Neonates with Abrupt Clinical Deterioration in Absence of Sepsis Risk Factors

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This study was conducted at the Neonatal Intensive Care Unit (NICU), Zagazig University Hospitals during a period of 20 months, aiming at increasing the awareness towards metabolic diseases among neonates with abrupt clinical deterioration in the absence of sepsis risk factors, hoping for early detection by using simple urine tests. The study included 115 newborns divided into: 95 newborns with abrupt clinical deterioration after a period of apparently healthy conditions and in the absence of sepsis risk factors and 20 healthy newborns as controls. Patients were subjected to full history taking, thorough clinical and neurologic examination and investigations that were done in NICU such as CBC, CRP, blood culture, serum electrolytes, blood glucose, arterial blood gases and serum ammonia. The following urine screening tests were done to all newborns: Ferric chloride test, nitrosonaphthol test, 2,4 dinitrophenyl hydrazine test, cyanide nitroprusside test, Benedict test, glucose oxidase and urinary ketones. Our results included +ve 2,4 dinitrophenyl hydrazine test in 51.6% of cases, +ve urinary ketones in 45.3% of cases, +ve ferric chloride test in 31.6% of cases, +ve Benedict test in 16.8% of cases, +ve cyanide nitroprusside test in 10.5% of cases, +ve nitrosonaphthol and glucose oxidase tests in 8% of cases. Three out of the 13 cases with sepsis had +ve urine tests, indicating that sepsis does not exclude screening for metabolic disorders. All urinary screening tests are -ve in the control group. We concluded that all neonates with abrupt deterioration of clinical conditions should be screened for metabolic disorders by simple urine tests which are rapid, easy and inexpensive, for early diagnosis and early treatment of these disorders with better outcome.

Key words: Screening, urine, metabolic disorders, sick neonates

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INTRODUCTION

In 1904, a British physician, Archibald E. Garrod described AlKaptonuria; a disease he classified as a lifelong congenital chemical alteration. Later on, in 1909, he described other diseases: Albinism, cystinuria and porphyria which he named Inborn Errors of Metabolism (IEMs). Garrods conclusions were completely correct in relation to the genetic basis of metabolic disorders and the gene-enzyme concepts (Dronamraju, 1998).

Inborn errors of metabolism cause hereditary metabolic diseases and classically they result from the lack of activity of one or more specific enzymes or defect in the transportation of proteins. The consequences can usually be the accumulation of substances normally present in small amount, the deficiency of specific final products; or critical intermediary products or furthermore the noxious excess of products of alternative pathways. The molecular basis is genetic mutations in enzymatic loci that affect activator proteins or co-factors for enzymes, protein transportation, carrier systems, or recognition markers (Wappner, 1993).

Recent advances in the diagnosis and treatment of inborn errors of metabolism have improved the diagnosis for many of these conditions. This makes it essential that the practicing pediatrician should be familiar with the clinical presentations of these disorders (Hoffmann, 2004).

Clinical features of metabolic disorders are non-specific and none of these features alone may point towards a metabolic disorder, so the main difficulty in diagnosing metabolic diseases lies in detecting which patients merit the detailed and costly laboratory investigations necessary to identify the biochemical defect. Therefore, it is advantages to develop a program of screening tests which can economically applied to large number of samples to detect abnormal metabolites (Menkes, 1995).

This study aimed at increasing the awareness towards metabolic diseases among neonates with abrupt clinical deterioration in the absence of sepsis risk factors and hoping for their early detection by using a set of rapid, easy and inexpensive simple urine tests.

MATERIALS AND METHODS

This study was conducted at the Neonatal Intensive Care Unit (NICU) in Zagazig University Hospitals, during a period of 20 months, from May 2005 to December 2006.

The study included 115 newborns, divided into two groups:

Group 1: Included 95 full term newborns, selected from NICU of Pediatric Zagazig University Hospital, with abrupt clinical deterioration in the form of convulsions, lethargy, respiratory distress, vomiting with or without dehydration, jaundice or coma, in the absence of sepsis risk factors and after a period of apparently healthy conditions.

Group 2: Included 20 healthy full term newborns as a control group.

Exclusion criteria:
- Prematurity.
- Risk factors of neonatal sepsis:
  - Premature rupture of the membrane > 12 h.
  - Maternal fever.
  - Chorioamnionitis.
  - Foul smelling of amniotic fluid.
- History suggestive of perinatal asphyxia:
  - Antepartum hemorrhage.
  - Maternal hypertension.
  - Delayed first breath or prolonged resuscitation
- Infant of diabetic mother.
  - All newborns were subjected to the following:
- Complete history taking with emphasis on neurological symptoms, consanguinity, similar condition in other family members and siblings with unexplained infant or neonatal death.
- Thorough clinical and neurological examination.
- Investigations that were done in NICU such as: Routine urine and stool analysis, complete blood count (CBC), C-reactive protein (CRP), blood culture, serum electrolytes (Na, K, Cl, Ca), arterial blood gases (ABG), blood glucose, liver and kidney functions and serum ammonia.
- Urine metabolic screening tests for all subjects:
  - Urinary pH, glucose, ketones, urobilinogen and bilirubin, using a commercial paper strips.
  - Benedict test (Thomas and Hawell, 1979).
  - Ferric chloride test (Shen and Abell, 1977).
  - Nitrosonaphthol test (Thomas and Hawell, 1979).
  - Cyanide nitroprusside test (Thomas and Hawell, 1979).
  - 2, 4 dinitrophenyl hydrazine test (Christenson and Azzazy, 1999).

Statistical methods: It was done by using SPSS (Statistical Package for Social Sciences) version 10 for

the year 1999. Data were expressed as number and percentage for qualitative variables, χ^2 (Chi-square) test for interpretation of the results. For comparing several tests against +ve clinical signs; we used Friedman test. For all statistical tests done, the threshold of significance is fixed at the 5% level (p-value).

RESULTS

Patients characteristics included male predominance in 69.5% of cases, positive family history in 9.5% of cases, with parental consanguinity in 76.8% of cases. Convulsions were the dominant symptom, presented in 44.2% of cases followed by lethargy in 42.1% then respiratory distress in 30.5%.

Blood culture and CRP were positive in 13.7% of cases, hyperammonemia in 42.1%, high anion gap in 27.4%, metabolic acidosis in 28.4% and respiratory alkalosis in 10.5% (Table 1).

Convulsions were generalized in 35 out of the 42 cases and subtle in 7 patients. Most patients with convulsions needed more than one anticonvulsant drugs to be controlled, 6 patients remained uncontrolled.

70.5% of patients had at least one positive urine screening test. Control group showed negative results for all urine screening tests. Patient's group showed positive 2,4 dinitrophenyl hydrazine in 49.5% of cases, positive ketones in 44.2%, positive ferric chloride test in 31.6%, positive cyanide nitroprusside test in 10.5%, positive Benedict test in 8.4%, positive nitrosonaphthol test in 7.4% and positive glucose oxidase in 1.1%. Three of our urine screening tests (2.4 dinitrophenyl hydrazine, ferric chloride; and urinary ketones) showed highly significant difference when comparing cases with control group (Table 2).

The dominant positive urine screening test in patients with convulsions was urinary ketones (69%), with lethargy was 2.4 dinirophenyl hydrazine (72%), with jaundice was Benedict test (85%), with vomiting were Benedict test (10%) and glucose oxidase test (10%), with respiratory distress was urinary ketone (86.2%) (Table 3). Three out of the 13 patients with positive blood culture and CRP had positive urine tests (2 with positive Benedict test and one with positive urinary ketones).

The dominant urine screening test in patients with hyperammonemia was 2.4 dinitrophenyl hydrazine (75%) and with high anion gap was urinary ketones (96.2%) (Table 3).

Table 1: Some clinical and laboratory data in our patients

Variables	No.	(%)
Positive consanguinity	73/95	76.8
Sex		
Male	66/95	69.5
Female	29/95	30.5
Similar condition in the family	9/95	9.5
Siblings with unexplained death	14/95	14.7
Convulsions	42/95	44.2
Lethargy	40/95	42.1
Jaundice	7/95	7.4
Vomiting	10/95	10.5
Respiratory distress	29/95	30.5
Hepatosplenomegaly	0/95	0.0
Positive blood culture	13/95	13.7
Positive CRP	13/95	13.7
High serum ammonia	40/95	42.1
High anion gap	26/95	27.4
ABG		
Metabolic acidosis	27/95	28.4
Respiratory alkalosis	10/95	10.5

Normal serum ammonia = up to 180 μmol L^{-1} in neonates. Normal anion gap [(Na$^+$ + K$^+$)-(Cl$^-$ + HCO$_3^-$)] = 8-16 mmol L^{-1}

Table 2: Urine metabolic screening tests

Parameters	Patients group +ve cases	(%)	Control group +ve cases	(%)	p-value
2,4 dinitrophenyl hydrazine	47/95	49.5	0/20	0	0.001
Cyanide nitroprusside	10/95	10.5	0/20	0	0.129
Nitrosonaphthol	7/95	7.4	0/20	0	0.210
Ferric chloride	30/95	31.6	0/20	0	0.003
Benedict	8/95	8.4	0.20	0	0.178
Glucose oxidase	1/95	1.1	0/20	0	0.645
Ketones	42/95	44.2	0/20	0	0.002

Table 3: Association between patients' characteristics and positive urine screening tests

Characteristics	Patients	2,4 dinitro-phenyl hydrazine	Cyanide nitroprusside	Nitroso-naphthol	Ferric chloride	Benedict	Glucose oxidase	Urine Ketones
Consanguinity	73/95	34/73 (46.6)	6/73 (8.2)	7/73 (28.8)	21/73 (28.8)	6/73 (8.2)	1/73 (1.4)	29/73 (39.7)
Same condition in family	9/95	4/9 (44.4)	0/9 (0.0)	0/9 (0.0)	4/9 (44.4)	1/9 (11.1)	0/9 (0.0)	6/9 (66.7)
Sibling with unexplained death	14/95	5/14 (35.7)	0/14 (0.0)	0/14 (0.0)	6/14 (42.9)	0/14 (0.0)	0/14 (0.0)	8/14 (57.0)
Convulsions	42/95	17/42 (40.5)	0/42 (0.0)	3/42 (7.1)	27/42 (64.3)	2/42 (4.8)	0/42 (0.0)	29/42 (69.0)
Lethargy	40/95	29/40 (72.0)	10/40 (25.0)	3/40 (7.5)	3/40 (7.5)	1/40 (2.4)	0/40 (0.0)	11/40 (27.5)
Jaundice	7/95	1/7 (14.3)	0/7 (0.0)	1/7 (14.3)	0/7 (0.0)	6/7 (85.7)	0/7 (0.0)	3/7 (42.9)
Vomiting	10/95	0/10 (0.0)	0/10 (0.0)	0/10 (0.0)	0/10 (0.0)	1/10 (10.0)	1/10 (10.0)	0/10 (0.0)
Respiratory distress	29/95	11/29 (37.9)	10/29 (34.0)	1/29 (3.4)	17/29 (58.6)	0/29 (0.0)	1/29 (3.4)	25/29 (86.2)
+ve blood culture	13/95	1/13 (7.7)	0/13 (0.0)	0/13 (0.0)	0/13 (0.0)	2/13 (15.4)	0/13 (0.0)	1/13 (7.7)
+ve CRP	13/95	1/13 (7.7)	0/13 (0.0)	0/13 (0.0)	0/13 (0.0)	2/13 (15.4)	0/13 (0.0)	1/13 (7.7)
↑Serum ammonia	40/95	30/40 (75.0)	10/40 (25.0)	3/40 (7.5)	3/40 (7.5)	0/40 (0.0)	0/40 (0.0)	11/40 (27.5)
↑Anion gap	26/95	11/26 (42.3)	10/26 (38.5)	1/26 (3.8)	17/26 (65.4)	0/26 (0.0)	0/26 (0.0)	20/26 96.2)

Values shown in parenthesis are in percentage

DISCUSSION

If the diagnosis of IEMs is reached through screening in a normal newborn, or early during the course of the disease, the therapeutic results will be extremely rewarding. This is evident in a study which reported that 30% of the patients with suspected IEMs had progressed to permanent neurological crippling. This has been disappointing, since approximately 80% of the diseases diagnosed have rewarding therapeutic measures (Pinar, 1998).

The initial step in the evaluation of any sick neonate and clearly the most important one is a thorough clinical assessment including a positive family history (Burton, 1998). This is evident in our study as there is positive family history of consanguinity in 76.8% of cases, siblings with unexplained neonatal death in 14.7% of cases and similar conditions in the family in 9.5% of cases.

The features of the clinical profile of our patients showed male predominance (69.5%) and parental consanguinity (76.8%). Another study reported that the features suggestive of metabolic disorders included a male predominance (76.6%) with consanguinity in 10% only (Sheffali *et al.*, 2000). The big difference in parental consanguinity which is high in our study is due to the high incidence of consanguineous marriages in Egypt. The male predominance can be explained by the fact that some IEMs are transmitted as X-linked recessive inheritance. The high incidence of IEMs among cases with positive family history of parental consanguinity is also observed by Vasant *et al.* (2001) and this can be explained by the fact that consanguineous marriages are more likely to produce offsprings affected by autosomal recessive disorders because relatives are more often share abnormal genes inherited from a common ancestor and most IEMs are autosomal recessive disorders.

In our study convulsions were the predominant symptom (44.2%), followed by lethargy (42.1%), respiratory distress (30.5%) and vomiting (10.5%). This comes in agreement with Lund *et al.* (2002) who reported that the predominant symptoms in neonates suggesting the possibility of metabolic disorders are convulsions and lethargy.

In our study most of seizures were intractable to therapy, about two thirds of patients required two or more anticonvulsant drugs and 6 of the 42 cases of convulsions were uncontrolled. This is in total agreement with Menkes (1995) who reported that convulsions are more likely to be uncontrolled in metabolic disorders.

Hyperammonemia was present in 42.1% of our cases, 77.5% of them had lethargy, 22.5% had metabolic acidosis and 2.5% had vomiting. Plasma ammonia level should be obtained for any child with unexplained vomiting, lethargy, or any other evidence of encephalopathy (Campistol *et al.*, 2005).

Respiratory distress was observed in 30.5% of our cases, it can be explained by the direct stimulatory effect of hyperammonemia on respiratory center and as a compensatory mechanism for metabolic acidosis.

Vomiting is a striking feature of many of the IEMs. It was evident in 10.5% of our cases. Many infants with IEMs have numerous formula changes and even have undergone surgery for pyloric stenosis before the diagnosis is finally established (Brandt, 1984).

Metabolic acidosis is one of the most striking features of IEMs. It was detected in 28.9% of our cases, 33.3% of them had increased anion gap. Metabolic acidosis with increased anion gap is typically present in organic acidemias, including propionic acidemia, methylmalonic acidemia and isovaleric acidemia, however the presence of renal tubular acidosis (metabolic acidosis with normal anion gap) does not exclude IEMs (Chakrapani *et al.*, 2002).

Three out of the 13 cases with +ve blood culture and +ve CRP (neonatal sepsis) had also +ve urine metabolic screening tests (two with +ve Benedict test and one with +ve 2,4 dinitrophenyl hydrazine test), indicating that the presence of neonatal sepsis does not exclude screening for IEMs. Sone IEMs (such as galactosemia) can predispose to gram negative septicemia (Chakrapani *et al.*, 2002), or both can co-exist (Ellaway, 2002).

Our urine screening tests showed a highly significant statistical difference between cases and controls as regard urine ketones, ferric chloride and 2,4 dinitrophenyl hydrazine tests. This is in agreement with a similar study made by Oliveira and Santos (2001).

2,4 dinitrophenyl hydrazine test was positive in 49.5% of our cases. This test indicates the presence of ketoacids in urine, therefore it is likely to be positive in phenylketonuria, tyrosinemia or histidinemia. A positive reaction is also given by acetone which may be anticipated in hyperglycinemia, isovaleric acidemia and glycogen storage disease type 1 and 3 (Christenson and Azzazy, 1999).

Cyanide nitroprusside test was positive in 10.5% of our patients. This test is searching for compounds with disulfite links, so it is positive in homocystinuria and cystinuria (Thomas and Hawell, 1979).

Nitrosonaphthol test was positive in 7.4% of our patients. This test gives positive results in tyrosine transaminase deficiency, tyrosinemia and transient tyrosinemia of the newborn. So positive results will guide us to search for tyrosine deaminase or tyrosinase enzymes abnormalities. Positive results also indicate

cysteine abnormalities e.g., homocystinuria, cystinuria, or cystinosis (Thomas and Hawell, 1979).

Ferric chloride test was positive in 31.6% of our patients. This test is non-specific and gives high positive results with multiple amino acids disorders and other metabolic disorders (Shen and Abell, 1977).

Benedict test was positive in 8.4% of our patients. This test searches for urine sugars and other reducing substances in urine. So, it is positive in diabetes mellitus, cystinosis, galactosemia, pentosuria, alkaptonuria and ascorbic acid (Thomas and Hawell, 1979).

Vasant *et al.* (2001) reported that manifestations of inherited metabolic disorders whether overt, life threatening or subtle were generally present in the neonatal period, so screening of sick neonate is stressed especially in those with positive urine ketones, as neonates do not produce ketones. This was evident in our study as 44.2% of our patients had positive urine ketones.

Urine ketones showed positive test results in 89.7% of cases presenting with respiratory distress, 69% with convulsions, 66.7% with similar condition in the family, 57.1% with siblings with unexplained neonatal deaths, 42.9% with jaundice, 39.7% with consanguinity and 30% with lethargy and hyperammonemia. This indicates that urine ketones test is a simple clue to the possibility of the presence of a metabolic error in a neonate presenting by any of the above complaints, especially organic acidemias, maple syrup urine disease and ketotic hyperglycinemia (James and Chase, 1974).

Since nitrosonapthol and cyanide nitroprusside tests can be used to screen very limited metabolic disorders, we can use them to diagnose specific metabolic disorders.

70.5% of our cases had presented a positive results in at least one of the urine screening tests. This is near to a similar study as using the same screening tests, who reported that 63.4% presented at least one positive test result (Olivera and Santos, 2001). But this is higher than a similar study done by Dave (2004) who reported that about 26% of high risk neonates with abrupt clinical deterioration were diagnosed with a metabolic disorder. This is may be due to using a more accurate chemical screening methods by gas chromatography and mass spectrometry. Also, some of our urine screening tests give positive results with many IEMs as ferric chloride test which gives positive results with phenylketonuria, tyrosinemia, maple syrup urine disease, histidinemia, alkaptonuria and hyperglycinemia.

CONCLUSION

This high positivity of these urine metabolic screening tests in these cases suggests a significant 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 practioners, pediatricians and neonatologists towards the metabolic disorders especially in places where more specific investigations are nearly unavailable.

RECOMMENDATIONS

- In the developing countries, where economic constraints limit the diagnosis of IEMs, it is recommended to provide these simple urine screening tests to broadly classify and identify IEMs.
- It is important to provide intensive cooperation between clinician, clinical chemist and the biochemist to select patients for screening program, to avoid unnecessary expensive investigations and to achieve a final molecular diagnosis.
- It is recommended to perform these screening tests early in the neonatal period as it is the time of substantial catabolism. Early diagnosis is important as many IEMs are treatable with successful outcome.
- Since IEMs are hereditary in nature, genetic counseling is recommended to the family of the patient. It is also recommended to avoid consanguineous marriages in this family.

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