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Blood Gases, Plasma Ammonia Levels and Urine Analysis; a Potential for Early Detection of Some Inborn Errors of Metabolism

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Authors' contributions

This work was carried out in collaboration between all authors. Authors THS, GO, AASS and WGA shared the study concept and design. Author AASS was responsible for patient selection. Authors MHH and AAY were responsible for data collection, obtained blood samples from the patients, carried out the whole biochemical assays, statistical analysis and managed the literature researches. All authors shared in data analysis and interpretation of results, manuscript writing and final editing. All authors approved the final revised draft.

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ABSTRACT

Aims: Inborn errors of metabolism (IEM) constitute a group of inherited disorders that cause significant morbidity and mortality. This diverse group of diseases present with different clinical manifestations that make the diagnosis a real challenge. The current work aimed to investigate the potential of some preliminary laboratory tests such as blood gases, plasma ammonia levels and urine analysis as rapid and conventional biochemical markers for early diagnosis of young children

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with high suspicion of metabolic disorders.
Methodology: The present study has been conducted on 50 patients suspected to have metabolic disorder according to the inclusion criteria selected from pediatric patients attending the pediatric, metabolic and neurological consultant clinics at the Pediatrics Department, Assiut university hospital, Egypt. In addition, to 20 healthy subjects were selected as the control group who had matched age and sex to the study group. All participants had their blood tested for complete blood count, gases, ammonia, electrolytes, urea, creatinine and glucose levels. Additionally, urine was tested for ketonuria, cysteinuria and fructosuria. Computed topography (CT) and cerebrospinal fluid (CSF) studies were also performed.
Results: There was a significant higher percent of consanguineous marriage among the patients' parents compared with the control group. Plasma ionized calcium, pO_2 and ammonia levels were significantly higher ($p < 0.05$), whilst, pCO_2, pH and HCO_3 were lower ($p < 0.05$) in patients versus the control group. One case had ketonuria and hyperglycemia but died before completing the investigatory workup. CT brain revealed that 60% of the included pediatric patients had brain atrophic changes. The final diagnoses of patients suspected to have metabolic disorders were: 11 (22%) had septicemia; 21 (42%) died before complete the final diagnosis; 12 (24%) suspected to have urea cycle defect and 6 (12%) suspected to have organic acidemia.
Conclusions: Blood gases, plasma ammonia levels and urine analysis are collectively simple and rapid laboratory tests that can give a preliminary indication for further investigations in pediatric patients with suspicious symptoms of metabolic disorders.

Keywords: Metabolic disorders; plasma ammonia; blood gases; urine analysis.

1. INTRODUCTION

Inborn errors of metabolism (IEM) constitute a group of inherited disorders that cause significant morbidity and mortality [1]. This diverse group of diseases present with different clinical manifestations that make the diagnosis a real challenge [1]. Early detection and appropriate investigations prevent complications and save lives [1]. IEM are mainly caused by genetic mutations and result in deficiency of an enzyme or a cofactor [2]. This usually leads to accumulation of a substrate that can't be metabolized by the deficient enzyme [2]. The resulting pathophysiological process leads to deficiency of a product that is essential for the physiological function of the cell [2]. The diagnosis of metabolic disorders depends on the subject's past medical history, detailed clinical examination and comprehensive laboratory investigations [3]. In this reference; serum ammonia, amino acids, acyl carnitine profile as well as some urinary organic acids would provide valuable diagnostic information [3]. Patients with IEM are occasionally very sick and may have significant morbidity and mortality if the diagnosis and appropriate management are delayed [4].

The initial presenting symptoms of most IEM are nonspecific such as poor feeding, vomiting and lethargy [1]. As sepsis presents in a similar way, it is sometimes difficult to differentiate sepsis from IEM [1]. The two conditions occasionally co exist [1]. It is reasonable to consider IEM in the differential diagnosis of unexplained illness especially severe sepsis in neonates [1]. Every newborn with unexplained neurological deterioration, hyperammonemia, metabolic acidosis or hypoglycaemia should be suspected of having an inherited error of intermediary metabolism [5]. Many of these conditions can be diagnosed clinically with the aid of simple laboratory investigations [5]. Since a substantial number of these diseases respond well to treatment but may otherwise be fatal, and in order to assure adequate prenatal diagnosis in subsequent pregnancies, a high index of suspicion and rapid diagnosis are necessary in the face of the clinical presentations described [5].

The objective of the present work was to investigate the possibility of using some preliminary laboratory tests as rapid and conventional biochemical markers in both blood and urine for early diagnosis of patients with high suspicion of metabolic disorder. This might help initiating the proper therapeutic interventions at early disease stages. Also to increase the awareness on this particular group of disease among the pediatricians in our country.

2. MATERIALS AND METHODS

2.1 Study Population

The present study was conducted on 50 patients with age range from 3 days to 5 years old, 56%

male and 44% female, who were attending the pediatric, metabolic and neurological consultant clinics at the Pediatrics Department, Assiut University Hospital, Egypt, after obtaining approval of university hospital ethics committee and informed consent from the parents of the included patients according to Good Clinical Practice (GCP/ICH) guidelines, during the study period from December 2011 to December 2013. In addition to 20 healthy subjects were selected as the control group who were age and sex matched to the study patients.

The study group inclusion criteria were [6,7]: children ≤ 5 years old with growth failure, failure to thrive, developmental delay, disturbed conscious level, seizures and encephalopathy, recurrent vomiting or diarrhea. Subjects were excluded from participation if their parents refused to participate, or were very critically ill, or proved to have another diagnosis.

2.2 Data Collection

The medical history and examination for all patients was taken according to pediatrics sheet, which was applied in the Pediatrics Department at Assiut University Hospital with special concern about the family history and whether the neonate was born to consanguineous parents or not. The previous history of older sibling miscarriage or genetically affected siblings was also checked, anthropometric measurements, attained physical and developmental milestones and careful clinical examination was done by both metabolic and neurological consultants. Furthermore, brain CT and CSF analysis were done for all included patients to exclude other diagnoses other than metabolic disorders such as meningitis, encephalitis, brain tumours, and intra-crainial hemorrhage or to confirm the presence of a metabolic disorder. The healthy subjects included in this study were selected according to the following inclusion criteria: They were selected to be matched as regard the age and gender with the patients and from the same geographic locality, not suffering from acute or chronic illness or chronically received any drugs or have family history of any metabolic or genetic disorders , and with no consanguineous marriage among their parents whom agree upon their participation in the present study. Syndromic children were also excluded.

2.3 Laboratory Workup

All study and control groups' participants were subjected to the following laboratory investigations; a 3cc of blood were collected by venipuncture and were divided into two tubes (preferably pre-chilled). The first 1.5 ml on EDTA as anticoagulant was used, for immediate estimation of complete blood count (CBC) and plasma ammonia (by using colorimetric method according to Anzalone et al. [8]) after being centrifuged at 3500 rpm for 15 minutes at 4℃ for the plasma to be separated. The other 1.5 ml of blood were put in a plain test tube for the estimation of serum sodium, potassium, ionized calcium, urea, creatinine and serum glucose. One ml of arterial blood sample was collected and used for blood gases analysis. This was obtained from radial or brachial or femoral arteries using a sterile needles and syringes to puncture the artery. The needle was advanced into the pulsating palpable artery until a blood flashback appears, then allow the syringe to fill to the appropriate level. The needle and syringe then withdrawn; a clean, dry piece of gauze or cotton wool was placed over the site and firm pressure for sufficient time was applied to stop the bleeding These syringes are pre-heparinized and handled to minimize air exposure that will alter the blood gas values and gently mixed, then the arterial blood gases were measured immediately by the automated blood gases analyzer using the whole anticoagulated blood without separation.

A clean, early morning urine sample was collected by a catheter which was the procedure of choice for neonates, infants and severely ill children. The urine sample for every included patient and was control evacuated in a clean container for immediate detection of ketonuria (detected chemically by Rothera test according to Alkonyl et al. [9]), cysteinuria (detected chemically by cyanide-nitroprusside test according to Milne et al. [10]) and reduced substances in urine (fructosuria) (detected chemically by Benedict's and Seliwanoff's tests [11,12].

2.4 Statistical Analysis

Analysis of data was done by IBM computer using SPSS (statistical program for social science version 12) as follows: Description of quantitative variables as mean ±SD. Description of qualitative variables as number and percentage. Chi-square test was used to compare the strength of association between quantitative categorical variables which was in the form of frequency, while, Mann-Whitney test was used to compare quantitative variables.

Correlation co-efficient test was used to rank variables positively or inversely. P value greater than 0.05 was considered statistically insignificant, while P value less that 0.05 was considered statistically significant, and P<0.001 was considered highly significant.

3. RESULTS

The consanguinity in the patient and control groups is presented in Table 1. A strong, significant statistical correlation between the consanguinity and study group compared to control group (p=0.000) was found.

Table 2 shows the mean hemoglobin and platelet count in the patient and control groups. A significant decrease in the hemoglobin and platelets in patient group compared to that in the control group was detected.

Mean serum electrolytes levels are shown in Table 3. No statistically significant differences in the serum sodium and potassium levels were found between the study and control groups. However, the ionized calcium was statistically higher in the patient group compared to the control group.

The blood gases (Table 4) show a significant statistical increase in pO_2 in the patients group compared to the controls group ($p < 0.001$). Whilst, there was a significant statistical decrease in pCO_2, pH and HCO_3 in the patients compared to control group ($p < 0.001$, $p < 0.05$ and $p < 0.001$, respectively).

Table 5 shows a statistically significant statistical increase in the plasma ammonia in the patients compared to the control (p=0.000), whereas, there was no significant difference in the blood glucose between the two groups.

On the other hand, the kidney function tests showed no difference between the patient and control groups. Regarding the urine analysis, there was one male case with positive ketonuria and hyperglycemia (150 mg/dl),this patient was one year old, and suffered from uncontrolled convulsion, shock and metabolic acidosis (pH=6.9,pO_2=170mmHg,pCO_2=4.8mmHg,HCO_3= 1mmol/l). There were no fructosuria and cysteinuria detected in both groups.

All patients had clear colorless CSF with no sediments except one male patient (4 days old) who had reddish discoloration of CSF (glucose=5.6 mmol/l, protein=10mg/dl). He was suffering from uncontrolled convulsions and disturbed conscious level, persistent metabolic acidosis and his blood gases showed that (PH=6,pO_2=170 mmHg, pCO_2=10 mmHg, HCO_3=4 mmol/l), The CT brain scan revealed no focal lesion. However, 30 infants (60%) in the study group had brain atrophy. The obstetric history in all cases revealed no significant abnormalities, but three cases had history of previous neonatal deaths and one stillbirth was reported by one case.

Table 1. The Consanguinity between patients and control groups

Consanguinity	Patients (n= 50)		Control (n= 20)		p-value*
	No.	%	No.	%	
Positive	45	90.0	0	0.0	0.000**
Negative	5	10.0	20	100.0	

**Chi-square test ** Statistical significant difference (p < 0.05)*

Table 2. Comparison of the mean hemoglobin and platelet counts among the studied groups

Complete blood picture	Study (n= 50)	Control (n= 20)	p-value*
Hb			0.000*
Mean ± SD	10.30 ± 2.41	12.68 ± 1.18	
Median	10.5	13.0	
Range	3.6 - 15.8	10.5 - 14.0	
Platelets			0.000*
Mean ± SD	196.04 ± 80.01	262.25 ± 53.80	
Median	200.0	278.5	
Range	36.0 - 450.0	140.0 - 330.0	

**Mann-Whitney test. ** Statistical significant difference (p< 0.05)*

Table 3. Serum electrolytes in patient and control groups

Electrolytes (mmol/l)	Patients (n= 50)	Control (n= 20)	P-value**
Sodium			0.984
Mean ± SD	139.06 ± 5.85	139.15 ± 3.76	
Median	139.5	140.0	
Range	123.0 - 152.0	135.0 - 146.0	
Potassium			0.249
Mean ± SD	4.21 ± 1.05	3.94 ± 0.41	
Median	4.2	3.9	
Range	2.4 - 7.9	3.5 - 4.6	
Calcium			0.024*
Mean ± SD	2.12 ± 2.90	0.97 ± 0.13	
Median	1.0	0.9	
Range	0.1 - 10.7	0.9 - 1.5	

***Mann-Whitney test. * Statistical significant difference (p< 0.05)*

Table 4. The blood gases in patients and controls

Blood gases	Patients (n= 50)	Control (n= 20)	p-value**
PO_2 (mmHg)			0.000*
Mean ± SD	126.42 ± 34.42	73.78 ± 3.79	
Median	131.0	73.5	
Range	70.0 - 177.0	70.0 - 80.0	
PCO_2 (mmHg)			0.000*
Mean ± SD	22.80 ± 10.33	39.11 ± 6.94	
Median	20.2	37.0	
Range	5.0 - 44.0	35.0 - 67.0	
pH			0.042*
Mean ± SD	6.54 ± 0.87	7.19 ± 0.16	
Median	6.7	7.3	
Range	5.0 - 8.0	7.0 - 7.4	
HCO_3 (mmol/l)			0.000*
Mean ± SD	12.20 ± 5.85	17.83 ± 0.94	
Median	11.0	18.0	
Range	1.0 - 23.2	17.0 - 20.0	

***Mann-Whitney test. * Statistical significant difference (p < 0.05)*

Table 5. The mean plasma ammonia and serum glucose levels in patients and controls

	Patients (n= 50)	Control (n= 20)	p-value**
Plasma ammonia: (µg/dl)			**0.000***
Mean ± SD	172.08 ± 93.36	89.75 ± 5.29	
Median	153.0	89.5	
Range	68.0 - 425.0	80.0 - 100.0	
Serum glucose: (mg/dl)			**0.156**
Mean ± SD	89.45 ± 15.69	93.30 ± 14.05	
Median	88.0	94.5	
Range	66.0 - 150.0	67.0 - 110.0	

***Mann-Whitney test. * Statistical significant difference (p< 0.05)*

Figs. 1, 2, 3 and 4 showed correlations between plasma ammonia and blood gases in the patient group.There was a significant positive correlation between plasma ammonia and pO_2 group (r=0.050 and p < 0.001). There was a significant inverse correlation between plasma ammonia, pCO_2, pH and HCO_3 in the patient group (r=-0.583, p < 0.001,r=-0.545, p < 0.001 and r =-0.580, p p < 0.001, respectively).

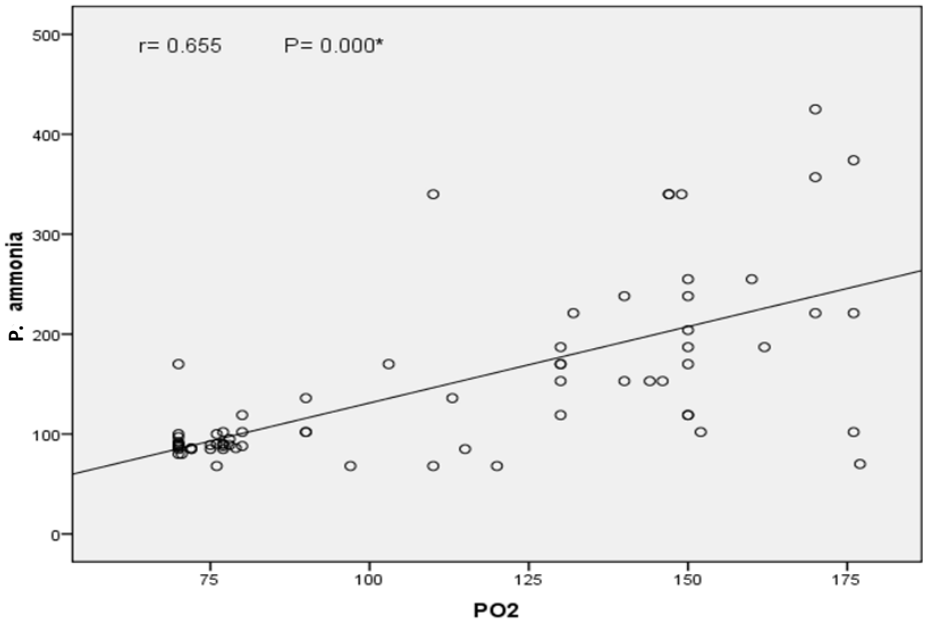

Fig. 1. Positive correlation between plasma ammonia and PO_2

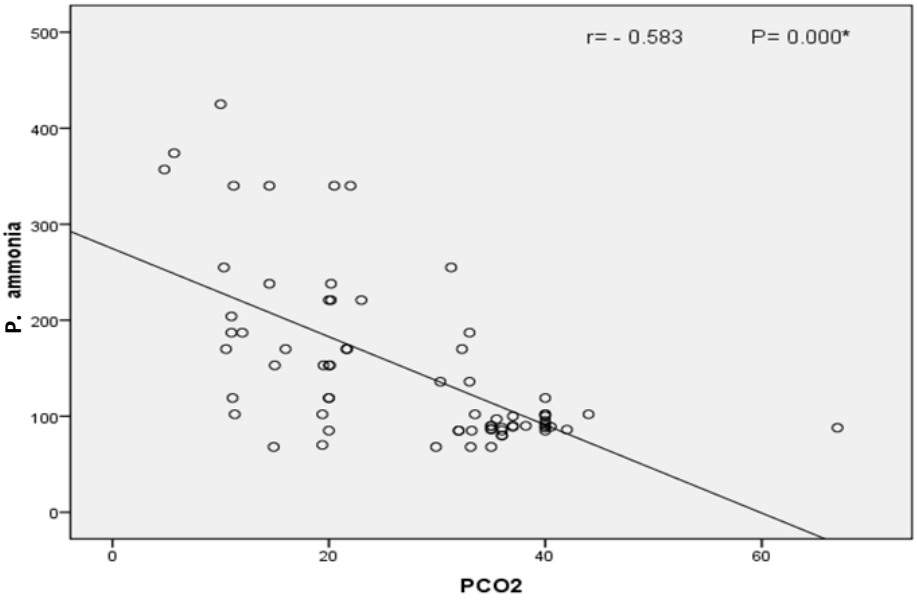

Fig. 2. Inverse correlation between plasma ammonia and PCO_2

The final diagnoses of the patients suspected to have metabolic disorders were: 11 (22%) had septicemia; 21 (42%) died before complete the final diagnosis; 12 (24%) suspected to have urea cycle defect and 6 (12%) suspected to have organic acidemia according to the amino acid assay profile results and require further confirmation of the diagnosis by performing mutation analysis of the suspected deficient enzyme gene.

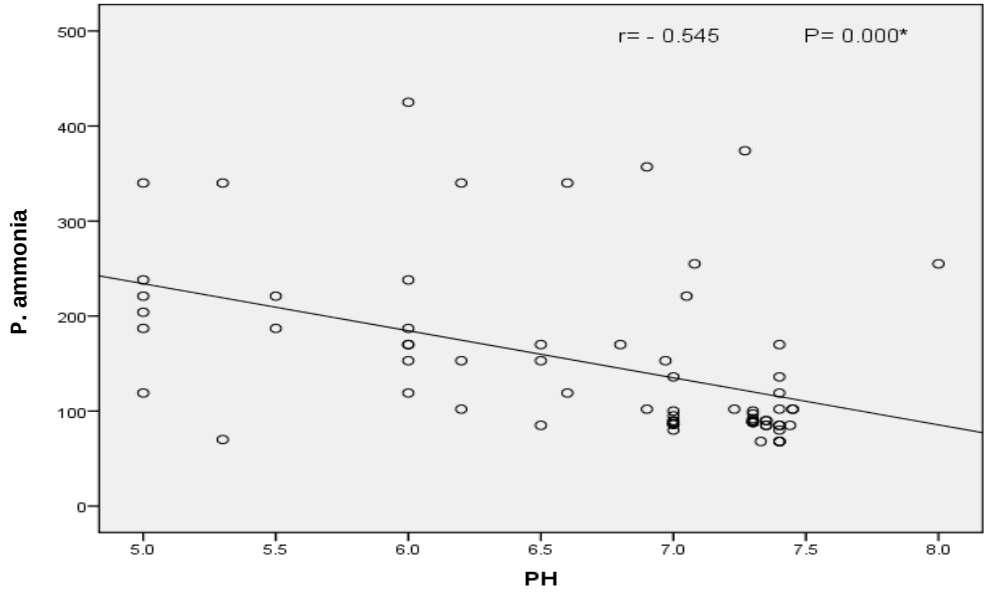

Fig. 3. Inverse correlation between plasma ammonia and pH

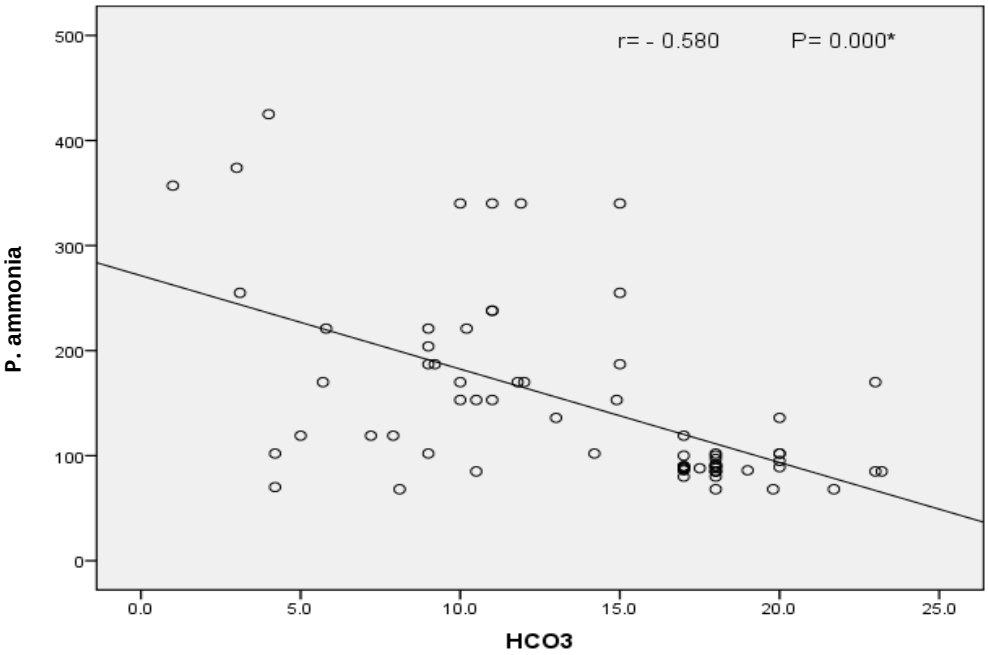

Fig. 4. Inverse correlation between plasma ammonia and HCO_3

4. DISCUSSION

Consanguineous marriages are recognized as being associated with higher risk for autosomal recessive diseases than in the general population [13]. Recent studies have shown that 20% to 50% of marriages in Arab countries are between relatives [14]. The rate was 68% in Egypt [15]. The present study showed significant higher consanguinity among the patients' parents

($p<0.001$) which agree with a study done by Al-Thihli et al. [16], their retrospective cross-sectional study was designed to evaluate the number of patients with IEM being followed at the only two tertiary centers in Oman treating such patients, and to calculate the consanguinity rates among these families. The electronic medical records of all patients were reviewed for demographic and clinical characteristics. The history of consanguinity was documented or available for 241 patients: 229 patients (95%) were born to consanguineous parents related as second cousins or closer [16].

The present study reported that there was significant increase in pO_2 and plasma ammonia in the patient group compared to the control group and there was a significant decrease in pCO_2, pH and HCO_3 in the patient group compared to the control group, which indicate the presence of metabolic acidosis among the patients suspected to have metabolic disorders. Our results showed that there was inverse correlation between serum ammonia, pCO_2, pH and HCO_3 in the patient group .These results agree with Iyer et al. [17], who studied the urea cycle disorders which are an important and treatable cause of hyperammonemia in the newborn and pediatric age group.

These results do agree also with Hudak et al. [18] and Ballard et al. [19], who studied hyperammonemia and proved that among the most important laboratory findings associated with inborn errors of metabolism presenting with an acute encephalopathy is hyperammonemia [18,19]. They concluded that plasma ammonia level should be obtained for any child with unexplained vomiting, lethargy, or other evidence of an encephalopathy [18,19]. Significant hyperammonemia is observed in a limited number of conditions [18,19]. Inborn errors of metabolism, including urea cycle defects and many of the organic acidemias are on the top of the list [18,19]. Additionally, in the differential diagnosis in the neonate there is a condition referred to as transient hyperammonemia of the newborn, whereas in the older infant, fatty acid oxidation defects may be considered [18,19]. The finding of marked hyperammonemia provides an important clue for diagnosis and indicates the need for urgent treatment to reduce the ammonia level [18,19].

The current study findings go also in line with Goldberg et al. [20] who studied metabolic acidosis as the second important laboratory feature of many of the inborn errors of metabolism. Metabolic acidosis was associated with an increased anion gap, readily demonstrable by measurement of arterial blood gases or serum electrolytes and bicarbonate [20] which agree with the current results.

Our results revealed that there was one male case with positive ketonuria and hyperglycemia, that patient was one year old and suffered from uncontrolled convulsion, shock and metabolic acidosis which was previously diagnosed as a diabetic patient then suspected to had metabolic disorder ($pH=6.9$,$pO_2=170$ mmHg, $pCO_2=4.8$ mmHg, $HCO_3=1$ mmol/l), but unfortunately died before completing the investigations required to reach final diagnosis. There was no fructosuria or cysteinuria detected in the patient and control groups. The CT and CSF studies were performed by pediatricians for the patients suffering from uncontrolled convulsions, disturbed conscious level and unexplained vomiting. All patients had clear, colorless CSF with no sediment, except a one male case 4 days old who had a reddish discoloration of his CSF (glucose=5.6 mmol/l,protein= 10mg/dl). He was suffering from uncontrolled convulsions and disturbed conscious level, there was persistent metabolic acidosis and his blood gases showed that ($pH= 6$,$pO_2=170$ mmHg,$pCO_2=10$ mmHg, $HCO_3=4$ mmol/l), the CT scan reviled no focal lesion, but unfortunately died before completing the investigations required to reach final diagnosis. The CT scan was done for patients suffering from uncontrolled convulsion, disturbed conscious level; there were 30cases with brain atrophy. Goldberg et al. [20] reported that infants with an inborn error of metabolism who present more abruptly with lethargy and poor feeding may first come to attention because of apnea or respiratory distress [20]. The apnea is typically central in origin and a symptom of the metabolic encephalopathy, but tachypnea may be a symptom of an underlying metabolic acidosis, as occurs in the organic academia [20]. Infants with urea cycle defects and evolving hyperammonemic coma initially exhibit central hyperventilation, which leads to respiratory alkalosis [20].

Regarding the final diagnoses of the included patients; septicemia has the highest frequency followed by urea cycle defect while organic academia had the lowest frequency. In disagreement with these findings, a study done by Multi et al. [21] on a total of 21 patients were admitted to Aga Khan University Hospital with

suspected congenital hyperammonemias who reported higher frequency of organic academia among those with congenital hyperammonemias and acidosis than urea cycle defect.

Urea which was is a nonspecific kidney function test showed significant increase in the patient group compared to the control group which may be due to hypovolemia which leads to decrease in renal blood supply in addition to stress of illness in these patients. Complete blood count showed that high significant statistical decrease in hemoglobin and platelets in the patient group compared to control group that may be due to poor nutrition in these chronically ill patients. There was a high significant statistical increase in ionized calcium in the studied metabolic disorder group compared to control group which may be due to intravenous infusion of calcium taken for cases suffering from convulsions.

5. CONCLUSIONS

Consanguinity, blood gases, plasma ammonia and urine analysis had a big role in the suspicion of some metabolic disorders particularly the fatal urea cycle defect and organic acidemia. The preliminary tests performed in this work should be done for infants aged from three days to five years suffering from any of the metabolic symptoms currently studied.

6. STUDY LIMITATIONS

The final diagnoses of some patients couldn't be reached due to death before completion of the investigatory battery while in some patients, molecular and genetic studies are required to confirm the diagnosis, which will be done in future studies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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