

Original Research Article**The potential of blood gases and plasma ammonia levels for early detection of pediatric patients having metabolic disorders****ABSTRACT**

Background: Metabolic diseases develop when the metabolic processes are abnormally functioning primarily due to the deficiency or accumulation of bio-chemical substances that might interfere with various normal physiological functions in the human body. Early diagnosis can lead to early medical and therapeutic interventions. The current work aimed to investigate the potential of some preliminary laboratory tests as a rapid and conventional biochemical marker for early diagnosis of young children with high suspicion of metabolic disorders.

Methods: Research Ethical approval was obtained for this investigational, two-grouped comparative study. The Study group inclusion criteria were children ≤ 5 years old with growth failure, failure to thrive, developmental delay, disturbed conscious level, seizures and encephalopathy. Otherwise, the children were enrolled in the Control group. The parents of all participants gave a written consent. Past medical history and clinical examinations were taken. All participants had their blood tested for complete blood count, gases, ammonia, electrolytes, urea, creatinine and glucose levels. Additionally, urine was tested for ketonuria, cystinuria and fructosuria. Computed topography (CT) and cerebrospinal fluid (CSF) studies were also performed.

Results: Plasma ionized calcium, pO_2 and ammonia levels were significantly higher ($p < 0.05$) in the study group. Whilst, pCO_2 , pH and HCO_3 were lower ($p < 0.05$). Urinalysis, CT

and CSF were comparable in the two groups ($p > 0.05$). Correlations among laboratory tests and study groups were determined.

Conclusion: Some simple and rapid laboratory tests can give a preliminary indication for further investigations in infants with suspicious symptoms of metabolic disorders.

Keywords: Metabolic diseases, plasma ammonia, blood gases, early diagnosis.

Introduction

Inborn problems of metabolism are usually referred to as congenital metabolic diseases[1]. The clinical presentations and complications of these metabolic diseases arise from the accumulation of bio-chemical substances that might interfere with various normal physiological functions in the human body that can affect its ability to synthesize or breakdown essential compounds[1]. The diagnosis of metabolic disorders depends on the subject's past medical history, detailed clinical examination and comprehensive laboratory investigations. In this reference; serum ammonia, amino acids, acyl carnitine profile as well as some urinary organic acids would provide a valuable diagnostic information[1].

Metabolic disorders such as Gaucher disease types 1 and 3, Niemann-Pick disease type B, carbohydrate, amino acid and fatty acid (e.g., lipidosis) metabolism disorders, organic acidemias and muco-polysaccharidoses, can be treated with diet or enzyme replacement therapy. However, many metabolic diseases have no cure. Allogeneic hematopoietic stem-cell transplantation from an appropriately-matched human leukocyte antigen (HLA)-matched donor might be considered medically necessary and has been proposed as a treatment option for several of these disorders. This transplantation can provide a constant source of enzyme replacement through the engraftment of the donor cells. In addition, the donor cells are not impeded by the blood-brain barrier allowing for enzyme delivery to the central nervous system[2]. However, hematopoietic stem-cell transplantation is currently recommended primarily for candidate children patients[3].

The objective of the present work was to investigate the possibility of using some preliminary laboratory tests as a rapid and conventional biochemical marker 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.

Methods

1. Subjects

A total of 70 patients participated in the current two-grouped research work. The study group consisted of 50 patients aged between 3 days and 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, during the period from December 2011 to December 2013. Whereas, the control group consisted of 20 patients who were attending to ophthalmology and ENT clinics who had matching age and sex to the study group.

The study group inclusion criteria were: children ≤ 5 years old with growth failure, failure to thrive, developmental delay, disturbed conscious level, seizures and encephalopathy. Subjects were excluded from participation if they had the previous inclusion criteria but their parents refused to participate, or were very critically ill, or proved to have another diagnosis. The Research Ethical Committee approval of Assiut University, Egypt was obtained and the parents/caregivers of all participants signed an informed consent form according to Good Clinical Practice (GCP/ICH) guidelines. Then, the participants were subjected to screening schedules questionnaire through assessment check lists according to their age groups which assessed clinically the attained developmental milestones up to 5 years of age, and whether delayed development was associated with any of the inclusion criteria[4].

The medical history and examination for all cases 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 for careful clinical examination was done by both metabolic and neurological consultants. Moreover, CT and CSF were done by Pediatric consultants whenever indicated i.e. for the patients suffering from uncontrolled convulsions, disturbed conscious level and unexplained vomiting.

2. Laboratory methods

All study and control groups' participants were subjected to the following laboratory investigations; a 3ml of blood were collected by venipuncture and were divided into two tubes (preferably pre-chilled). The first 1.5ml on EDTA as anticoagulant, for immediate estimation of complete blood count (CBC) and plasma ammonia after being centrifuged at 3500 rpm for 15 minutes at 4°C 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. Blood gases were also determined. 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 patient and control was evacuated in a clean container for immediate detection of ketonuria, cystinuria and reduced substances in urine (fructosuria). The results of the laboratory tests of both the study and control groups were then statistically compared.

3. Statistics

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 $\pm$ SD. Description of qualitative variables as number and percentage. Chi-square test was used to compare qualitative variables, while, Mann-Whitney test was used to compare quantitative variables. Correlation co-efficient test was used to rank variables positively or inversely. P value >0.05 insignificant, $P<0.05$ significant, $P<0.001$ highly significant.

Results:

The consanguinity in the study 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 (1): The Consanguinity between study and control groups

Consanguinity	Study (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 shows the complete blood picture in the study and control groups. A significant statistical decrease in the hemoglobin and platelets in study group compared to that in the control group ($p=0.000$) was detected.

Table (2): The Complete blood picture between study and control groups

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

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

The serum electrolytes are shown in table 3. No 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 studied metabolic disorder group compared to the control group ($p=0.024$).

Table (3): Serum electrolytes in study and control groups.

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

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

The blood gases (table 4) show a significant statistical increase in pO_2 in the study group compared to the control group ($p=0.000$). Whilst, there was a significant statistical decrease in pCO_2 , pH and HCO_3 in the study compared to control group ($p=0.000$, 0.042 and 0.000, respectively).

Table 5 shows a significant statistical increase in the plasma ammonia in the study group compared to the control group ($p=0.000$), 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 study 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 ($pH=6.9$, $pO_2=170$ mmHg, $pCO_2=4.8$ mmHg, $HCO_3=1$ mmol/l). There were no fructosuria and cystinuria 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,pO₂=170mmHg,pCO₂=10 mmHg,HCO₃=4mmol/l),The CT brain scan reviled no focal lesion. However, 30 infants in the study group had brain atrophy. The obstetric history to all cases reviled no significant abnormalities, except three cases had history of previous neonatal deaths and one stillbirth was reported by one case.

Table (4): The blood gases in study and control groups.

Blood gases	Study (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)

Figures 1, 2, 3 and 4 show a correlation between serum ammonia and blood gases in the study group. There was a significant positive correlation between serum ammonia and pO_2 group ($r=0.655$ and $p=0.000$). There was a significant inverse correlation between serum ammonia, pCO_2 , pH and HCO_3 in the study metabolic disorder group ($r=-0.583$, $p=0.000$, $r=-0.545$, $p=0.000$ and $r=-0.580$, $p=0.000$, respectively).

Table (5): The plasma ammonia and blood glucose in patient and control groups.

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

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

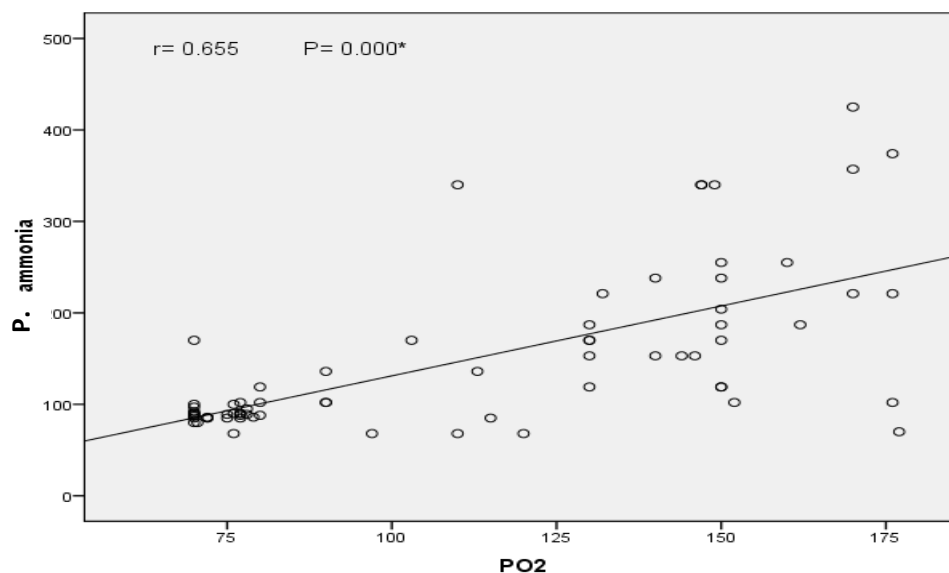


Figure (1): Correlation between Plasma ammonia and PO_2

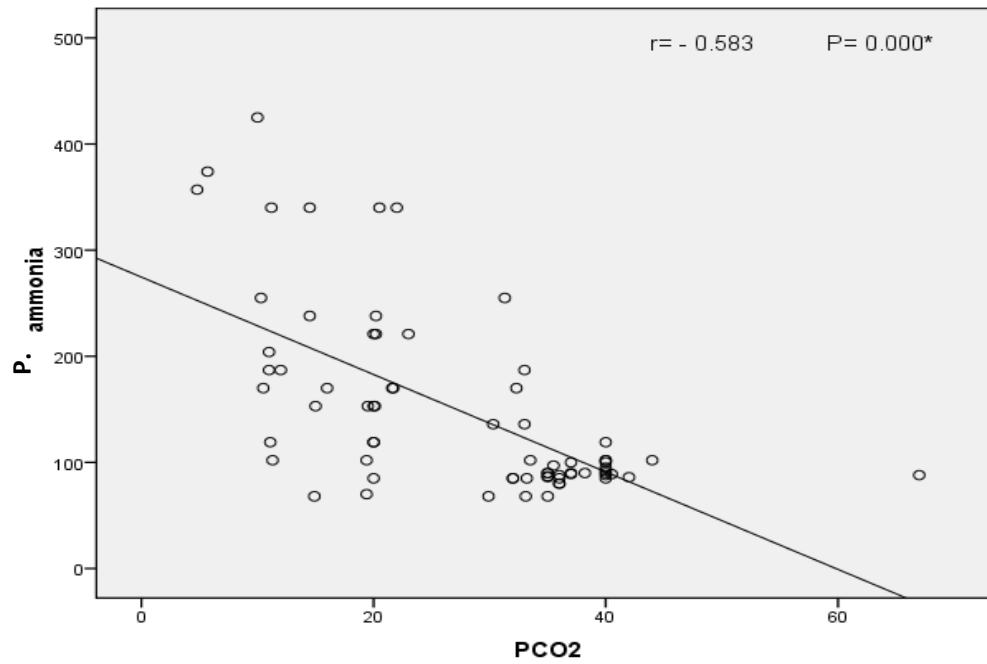


Figure (2): Correlation between plasma ammonia and PCO₂

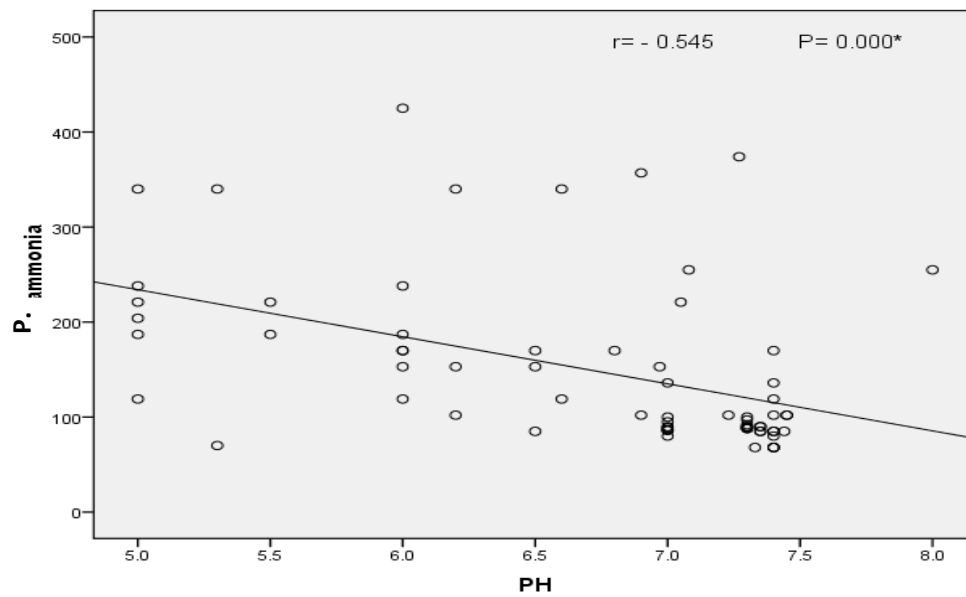


Figure (3): Correlation between plasma ammonia and pH

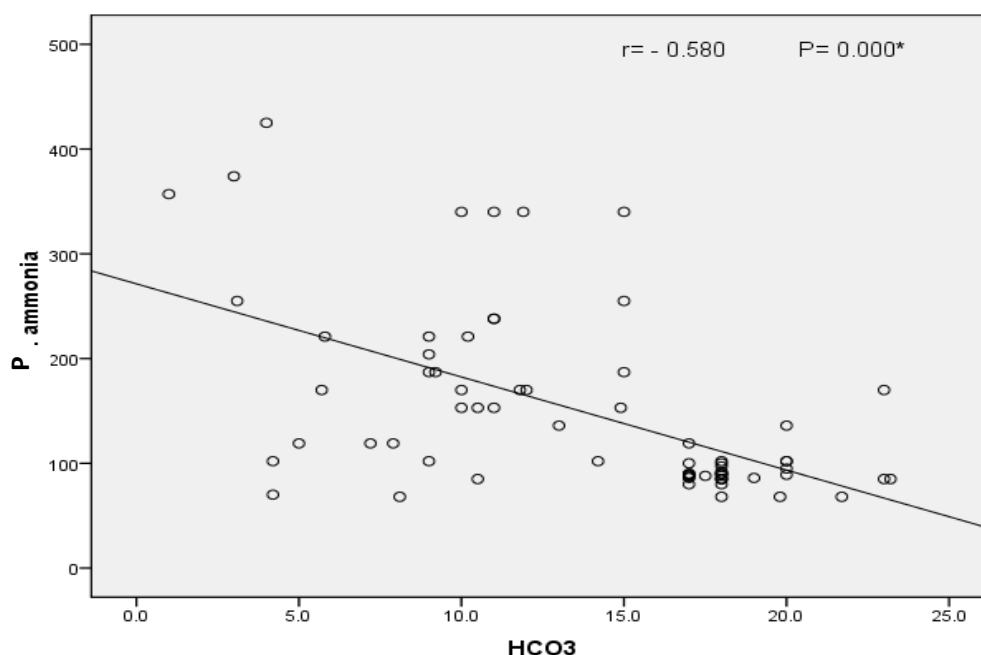


Figure (4): Correlation between plasma ammonia and HCO_3

Discussion

Every newborn with unexplained neurological deterioration, hyperammonemia, metabolic acidosis or hypoglycaemia should be suspected of having an inherited error of intermediary metabolism. Many of these conditions can be diagnosed clinically with the aid of simple laboratory investigations. 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].

Diagnosis of metabolic disorders depends on a careful history, detailed clinical examination and request of relevant investigations. Important points in the history include the age of onset of symptoms, developmental delay with or without regression, history of consanguinity, decompensation related to change of diet and vaccination and blood gases. If the suspicion of metabolic disorder is still high, serum ammonia, amino acids, acylcarnitine profile and urine organic acid would provide valuable diagnostic information [1].

The present study showed significant statistical correlation between consanguinity and the studied metabolic disorder group ($p=0.000$) which agree with [6] who studied the correlation between consanguinity and metabolic disorder. Their samples were chorionic villus tissue or amniotic fluid and concluded that most of the metabolic disorders were single gene defects, inherited in an autosomal recessive manner, with a 25% recurrence risk. Therefore when the diagnosis is known and confirmed in the index case, prenatal diagnosis can be offered, wherever available for the subsequent pregnancies.

William et al., (2008) studied the offspring of consanguineous relationships and concluded that they were at a greater risk of certain genetic disorders [7]. Autosomal recessive disorders occur in individuals who are homozygous for a particular recessive gene mutation. This means that they carry two copies (alleles) of the same gene. Except in certain rare circumstances (new mutations or uniparental disomy) both parents of an individual with such a disorder will be carriers of the gene these results agree with the current study.

Benjamin P (2010) studied the carriers and concluded that they are not affected and will not display any signs that they are carriers, and so may be unaware that they carry the mutated gene [8]. As relatives share a proportion of their genes, it is much more likely that related parents will be carriers of an autosomal recessive gene, and therefore their children are at a higher risk of an autosomal recessive disorder this result agreed with our current study.

Moreover, Kinsdon HM (2002) reported that the extent to which the risk increases depends on the degree of genetic relationship between the parents; so the risk is greater in mating relationships where the parents are close relatives, but for relationships between more distant relatives, such as second cousins, the risk is lower although still greater than the general population [9].

The present study reported that there was significant statistical increase in pO_2 in the studied metabolic disorder group compared to the control group ($p=0.000$) and there was a significant statistical decrease in pCO_2 , pH and HCO_3 in the studied metabolic disorder group compared to the control group ($p=0.000$, 0.042 and 0.000 , respectively). There was also a significant statistical decrease in pH in the studied metabolic disorder group compared to the control group ($p=0.000$). Our results showed that there was inverse correlation between serum ammonia, pCO_2 , pH and HCO_3 in the studied metabolic disorder group ($r=-0.583$, $p=0.000$, $r=-0.545$, $p=0.000$ and $r=-0.580$, $p=0.000$, respectively). These results agree with Iyer et al. (2012), who studied the urea cycle disorders which are an important and treatable cause of hyperammonemia in the newborn and pediatric age group [10]. The presentation in adolescence or adult life is rare and can manifest as frequent vomiting and behavioral changes. An inherited metabolic disorder should be considered in adults with obvious or occult encephalopathy which agree with the current results showing a convulsion and disturbed conscious level in the studied patient group. Delays in the diagnosis and treatment may lead to irreversible neuronal damages.

These results do agree also with Hudak et al. (1985) [11] and Ballard et al. (1978) [12], 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. They concluded that plasma ammonia level should be obtained for any child with unexplained vomiting, lethargy, or other evidence of an encephalopathy. Significant hyperammonemia is observed in a limited number of conditions. Inborn errors of metabolism, including urea cycle defects and many of the organic acidemias are on the top of the list. 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. The finding of marked hyperammonemia provides an

important clue for diagnosis and indicates the need for urgent treatment to reduce the ammonia level.

The current study findings go also in line with Goldberg et al. (1979), 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 [13] which agree with the current results as the blood gases in the patient group were pO_2 , pCO_2 , pH and HCO_3 (126.42 ± 34.42 , 22.80 ± 10.33 , 6.54 ± 0.87 and 12.20 ± 5.85 , respectively) compared to the control group which were 73.78 ± 3.79 , 39.11 ± 6.94 , 7.19 ± 0.16 and 17.83 ± 0.94 , respectively.

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 have metabolic disorder ($pH=6.9$, $pO_2=170$ mmHg, $pCO_2=4.8$ mmHg, $HCO_3=1$ mmol/l). There was no fructosuria or Cystinuria 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=10 mg/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 revealed no focal lesion. The CT scan was done for patients suffering from uncontrolled convulsion, disturbed conscious level; there were 30 cases with brain atrophy. Goldberg et al. (1979) 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[13]. 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 acidemias. Infants with urea cycle defects and evolving hyperammonemic coma initially exhibit central hyperventilation, which leads to respiratory alkalosis.

Urea which was 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.

In conclusion, Consanguinity, blood gases and plasma ammonia had a big role in the suspicion of some metabolic disorders. 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.

References

- [1] Fernandes J, Saudubray J-M, Van Den Berghe G et al. Inborn metabolic diseases: Diagnosis and treatment: Springer Science & Business Media; 2006.
- [2] Prasad VK, Mendizabal A, Parikh SH, Szabolcs P, Driscoll TA, Page K, Lakshminarayanan S, Allison J, Wood S, Semmel D, Escolar ML, Martin PL, Carter Sand Kurtzberg J: Unrelated donor umbilical cord blood transplantation for inherited metabolic disorders in 159 pediatric patients from a single center: Influence of cellular composition of the graft on transplantation outcomes. *Blood*. 2008; 112(7): 2979-2989.
- [3] Clarke LA and Heppner J: Mucopolysaccharidosis type i. 2011.
- [4] Chopra S, Brendel V, Zhang JB, Axtell JD and Peterson T: Molecular characterization of a mutable pigmentation phenotype and isolation of the first active transposable element from sorghum bicolor. *Proceedings of the National Academy of Sciences of the United States of America*. 1999; 96(26): 15330-15335.

- [5] Aleck KAand Shapiro LJ: Genetic-metabolic considerations in sick neonate. *Pediatric Clinics of North America*. 1978; 25(3): 431-451.
- [6] Rimoin DL, Connor JM, Pyeritz REand Korf BR. *Emery and rimoin's principles and practice of medical genetics*: Churchill Livingstone Elsevier; 2007.
- [7] Marshall WJand Bangert SK. *Clinical biochemistry: Metabolic and clinical aspects*: Elsevier Health Sciences; 2008.
- [8] Pierce BA. *Genetics: A conceptual approach*: Macmillan; 2010.
- [9] Kinsdon H: *Abc of clinical genetics*. London: BMJ Publishing Group; 2002.
- [10] Iyer H, Sen M, Prasad C, Rupar CAand Lindsay RM: Coma, hyperammonemia, metabolic acidosis, and mutation: Lessons learned in the acute management of late onset urea cycle disorders. *Hemodialysis International*. 2012; 16(1): 95-100.
- [11] Hudak ML, Jones MDand Brusilow SW: Differentiation of transient hyperammonemia of the newborn and urea cycle enzyme defects by clinical presentation. *Journal of Pediatrics*. 1985; 107(5): 712-719.
- [12] Ballard RA, Vinocur B, Reynolds JW, Wennberg RP, Merritt A, Sweetman Land Nyhan WL: Transient hyper-ammonemia of preterm infant. *New England Journal of Medicine*. 1978; 299(17): 920-925.
- [13] Goldberg RN, Cabal LA, Sinatra FR, Plajstek CEand Hodgman JE: Hyper-ammonemia associated with perinatal asphyxia. *Pediatrics*. 1979; 64(3): 336-341.