e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(6); 14-18

Original Research Article

Utility of Urine Metabolic Screening Test to Diagnose Spectrum of Inborn Metabolic Disorders in Jaipur, Rajasthan

Rupesh Kumar[1], Suresh Meena[2], Kailash Meena[3], Shakuntala Saini[4]

[1]Assistant Professor, Department of Biochemistry, National Institute of Medical Sciences and Research Centre, Jaipur, Rajasthan
[2]Associate Professor, Department of Biochemistry, National Institute of Medical Sciences and Research Centre, Jaipur, Rajasthan
[3]Senior Professor, Department of Paediatrics, SMS Medical College and Attached Hospital, Jaipur, Rajasthan
[4]Senior Professor, Department of Biochemistry, SMS Medical College and Attached Hospital, Jaipur, Rajasthan

Received: 20-03-2023 / Revised: 11-04-2023 / Accepted: 06-05-2023
Corresponding author: Rupesh Kumar
Conflict of interest: Nil

Abstract

Enzyme defect in biochemical or metabolic pathways leads to development of Inborn metabolic Disorders (IMDs) which are rare genetic or inherited disorders. Defect in biochemical or metabolic pathways alters proteins, fats and carbohydrates metabolism or impaired organelle function presenting as complicated medical conditions. IMDs involve great complexity of the underlying pathophysiology, biochemical workup, and molecular analysis, and have complicated therapeutic options for management IMDs lead to the physical and mental disability and death of infants, which can be prevented if detected early. Early detection of IMDs relies on a high index of clinical suspicion and co-ordinated access to specialized laboratory services So, the aim and objective of present study was to diagnose suspected IEM by using simpler and convenient urine biochemical tests 398 cases out of 512 referred cases of IEM suspicion showed the diagnostic pattern IMDs which was clinically correlated confirmed by the clinician who referred. Hence these parameters can be added as screening markers differentiate and diagnose IMDs.

Keywords: Inborn Metabolic Disorders, Urine Metabolic Tests.

Introduction

Inborn errors of metabolism (IEM) are group of disorder which arises due to defect in single gene which produce defective or nonfunction enzyme that affect the metabolic pathways. Defected metabolic pathways leads to block in the normal metabolic pathway which in turn leads to the toxic accumulation of the intermediates or deficiency of essential intermediates. Accumulation or deficiency of these intermediates reflects in the physical and mental health of the infants, if early treatment not escalated

It is important for paediatricians and neonatologists to suspect IMDs based on physical sign and symptoms as a cause of illness in the neonatal period. Many IMDs are treatable and in most cases, successful outcome is dependent on a rapid diagnosis and early instigation of therapy.

In desperately sick neonates for whom no diagnosis is readily available, New born screening is near the top of the list of differential diagnoses. IEMs can present in the new born in a variety of ways. Typically, an IEM is suspected as a result of a suggestive combination of acute clinical symptoms without any prior warning.

Early detection of IEM is based on the synergy between clinical suspicion and co-ordinated access to specialized new born screening (NBS) services. NBS forms the basis of the final confirmed diagnosis in several IMDs. NBS investigations are of different categories

1. General metabolic screening tests
2. Specific metabolite assays
3. Enzyme studies
4. DNA analysis

There are different types of IEM disorders, to name a few-amino acid disorders, urea cycle disorders, disorders of carbohydrate metabolism, Lysosomal storage, and mitochondrial disorders. Most of the symptoms of these disorders like poor feeding, vomiting, failure to thrive, developmental delay and diarrhoea overlap with other IEM and also non-IEM disorders.

This makes the first line of suspicion for IEM difficult. The other challenge is availability and viability of worldwide accepted diagnostic modalities like tandem mass spectra techniques; whole genome sequencing and specific enzyme assay to screen and confirm IEM

In India where the concept of mass newborn screening for IEM disorder is not a standard operational protocol [1]. There are very limited diagnostic laboratories in India which offer confirmatory tests for IEM disorders using mass spectrometry technique and specific enzyme activity assays in comparison to huge live births in India (19.3 births/1000 population, 2016 est.), the burden of which obviously delay the turnaround time for confirmation of the disease including the pre and post-analytical error as an added problem to it.

Some of the treatable IEM disorders like Phenylketonuria, Galactosemia, and mitochondrial disorders require immediate intervention otherwise lead to permanent disability or fatal consequences.

In this study our focus is to early diagnosis of IMDs using basic techniques so that the clinical intervention can be started well before time to avoid irreversible damage.

So, we did preliminary NBS for suspected cases of IMDs by measuring blood levels of ammonia, lactate, pyruvate and amino acids.

End product of glycolytic pathway are pyruvate and lactate. These play important role in energy production and utilization of NADH + H+ and NAD+. Thus, concentrations of these redox couple in blood reflect the nutritional and cytosol oxido-reduction status of an individual.

Hyper ammonia

When liver function is compromised, due either to genetic defects of the urea cycle or liver disease, blood levels can rise above 1,000 micro mol/L. Such hyper ammonemia is a medical

emergency, because ammonia has a direct neurotoxic effect on the CNS. For example, elevated concentrations of ammonia in the blood cause the symptoms of ammonia intoxication, which include tremors, slurring of speech, somnolence, vomiting, cerebral edema, and blurring of vision. At high concentrations, ammonia can cause coma and death.

Congenital hyperammonaemia:

Genetic deficiencies of each of the five enzymes of the urea cycle causes congenital Hyperammonemia. Ornithine transcarbamoylase deficiency, which is X-linked, is the most common of these disorders, predominantly affecting males,

although female carriers may become symptomatic. All of the other urea cycle disorders follow an autosomal recessive inheritance pattern. In each case, the failure to synthesize urea leads to hyperammonemia during the first weeks following birth. Historically, urea cycle defects had high morbidity (neurological manifestations) and mortality. Treatment included restriction of dietary protein in the presence of sufficient calories to prevent catabolism. Administration of compounds that bind covalently to amino acids, producing nitrogen-containing molecules that are excreted in the urine, has improved survival. For example, phenylbutyrate given orally is converted to phenylacetate. This condenses with glutamine to form phenylacetylglutamine, which is excreted

Hyperammonemia is an important laboratory finding associated with IEM disorder However, normal ammonia level doesn't exclude the absence of IEM disorder.

Concentrations of various amino acids in plasma depend on the metabolic state of the individual and thus an altered plasma amino acid profile is a metabolic consequence of impairments in the electron transport chain in mitochondria and associated amino acid metabolism [2, 3].

Since the parameters mentioned has their role in overlapping symptoms of IEM from non-IEM disorder, the objective of this study is to find the characteristic pattern of all three parameters: L/P ratio, Ammonia and amino acid profile in differentiating IEM disorders particularly mitochondrial and respiratory chain abnormalities from non IEM disorders.

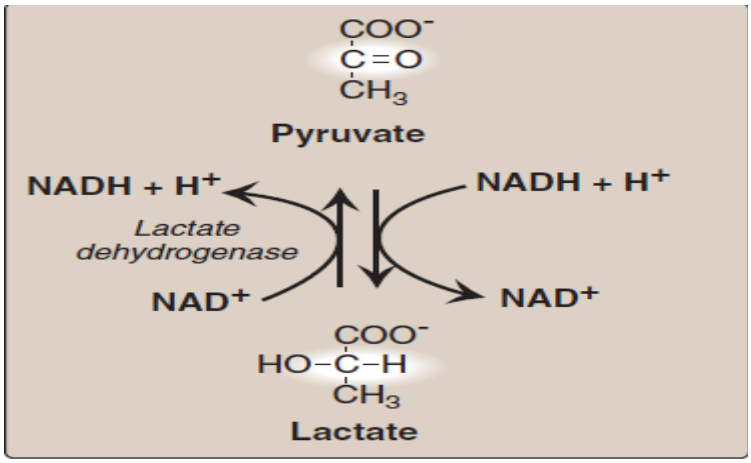

Figure 1: Interconversion of lactate and Pyruvate

Materials and Methods

This prospective experimental study was conducted in Department of Biochemistry, SMS Medical College and attached Hospital, Jaipur India. Institutional ethical clearance was obtained for the study.

Study Group

Group 1: Patients which has normal levels of blood ammonia, pyruvate and lactate.

Group 2: Patients which has High levels of blood ammonia and normal pyruvate and lactate level.

Group 3: Patients which has High levels of blood ammonia and High levels of pyruvate and lactate level.

Group 4: Patients which has normal blood ammonia and high pyruvate and lactate level.

Result

The median values and the interquartile ranges for different parameters is shown in Table 1.

Table 1: Biochemical parameters in different groups

Parameter	Normal Value	Group 1	Group 2	Group 3	Group 4
Ammonia	20–80 μg/dl	20 (25,45)	90(95,115)	96(87,191)	38(20,48)
Lactate	14–20 mg/dl	9.7(8,14)	15(11,14)	25(14,50)	20(16,28)
Pyruvate	0.3–2 mg/dl	0.3(0.3,0.6)	0.9(0.6,0.8)	0.3(0.2,0.44)	0.4(0.3,06)
L/P Ratio	17-22	22(19,22)	17(13,19)	82(45,172)	58(45,85)
Mann-Whitney U test, Median values with IQR1 & IQR2					

Discussion

Substrate levels of Cytoplasmic NADH/NAD ratio gives the direction for the conversion of lactate to pyruvate and help us to diagnose cytosolic redox status [5]. Deficiency of pyruvate dehydrogenase complex enzyme or respiratory chain dysfunction leads to accumulation of pyruvate. Large amount of pyruvate will be diverted for the formation of lactic acid or alanine production leading to lactic acidosis and hyperalaninaemia.

In some instances, increased blood lactate is seen in spite of fully functional PDH complex in case of respiratory chain dysfunction. The increased rate of anaerobic Glycolysis taking place in cytosol and subsequent compromise of energy metabolism will increase the NADH/NAD-ratio and thus elevated lactate levels in cytosol which mirror the elevated L/P ratio in blood

Conclusion

Samples of suspected IEM cases (Total 596) which was received in lab for routine investigations were taken for the study after their analysis. Of the total samples, 385 samples showed elevated lactate pyruvate ratios (group 3 and 4) in plasma, 211 cases showed elevated lactate levels in plasma which is a nonspecific marker of mitochondrial disease as reported by Haas et al. [7, 8] and 85 out of these 211 elevated lactate populations are found to fall into hyperalaninaemia category following characteristic pattern in amino acid profile supporting the hypothesis of abnormal increase in plasma alanine levels in case of mitochondrial disease.

References

1. Hannon WH, Grosse SD. Using tandem mass spectrometry for metabolic disease screening among newborns. Morb Mortal Wkly Rep. 2001;50:1–22.
2. Vassault A. Lactate, pyruvate, acetoacetate and 3-hydroxy butyrate. In: Blau N, Duran M, Gibson KM, editors. Laboratory guide to the methods in biochemical genetics. Heidelberg: Springer; 2008. p. 35–50.
3. Gibson KM, Jacobs C. Investigation for metabolic diseases: biochemical studies. In: Hoffman GF, Zschocke J, Nyhan WL, editors. Inherited metabolic diseases: a clinical approach. Heidelberg: Springer; 2010. p. 263–80.
4. Babu SVS, Shareef MM, Shetty APK, Shetty KT. HPLC method for amino acids profile in biological fluids and inborn metabolic disorders of aminoacidopathies. Indian J Clin Biochem. 2002;17:7–26.
5. Touati G, Mochel F, Rabier D. Diagnostic procedures: functional tests and post-mortem protocol. In: Saudubray JM, Van den Berghe G, Walter JH, editors. Inborn metabolic diseases. Diagnosis and treatment. 5th ed. Heidelberg: Springer; 2012; 88–95.
6. Das AM, Steuerwald U, Illsinger S. Inborn errors of energy metabolism associated with myopathies. J Biomed Biotechnol. 2010;2010:19.
7. Haas RH, Parikh S, Falk MJ, Russell P, Wolf NI, Darin N, et al. The in-depth evaluation of suspected mitochondrial

disease. MolGenet Metab. 2008;94:16–37.
8. Kamath S, Bhaskaranand N, Rao A. Spectrum of clinical symptoms in children with elevated lactate: pyruvate ratio in a tertiary care setting. Asian J Biomed Pharm Sci. 2014;4:34–8.

.