



Screening of Aminoacidurias in Children

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ABSTRACT

Inborn errors of metabolism (IEM) are genetically determined biochemical disorders due to specific congenital defects in protein molecules. In infants and young children the inborn errors produce mental deficiency and serious ill health. Aminoacidurias being one of the common causes of preventable mental retardation, mass screening helps in early detection and early intervention to prevent disability and mortality. Thus the present study was undertaken to screen children below 5 yrs of age showing signs and symptoms of aminoacidurias. 604 children with such symptoms were screened by basic urine screening tests, of which three were found to be positive. The urine and plasma samples of these 3 children were subjected to thin layer chromatography (TLC) and confirmed by high performance liquid chromatography (HPLC). They were found to be suffering from phenylketonuria (PKU). Hence urine screening tests, TLC followed by HPLC are rapid and convenient methods for early diagnosis of aminoacidurias in children.

Keywords- *Inborn errors of metabolism (IEM), Aminoacidurias, Thin layer chromatography (TLC), High performance liquid chromatography (HPLC) and Phenylketonuria (PKU).*

INTRODUCTION

Metabolic disorders also called as inborn errors of metabolism are genetically determined biochemical disorders due to specific congenital defects in the structure or function of protein molecules¹. A substantial number of genetically determined biochemical disorders in infants and young children produce mental deficiency and serious ill health in early life². Diseases of amino acid metabolism are the common disorders and have been the frequent

target of mass screening programmes because many of the available screening methods were designed to detect biochemical imbalance in amino acids in physiological fluids.

According to WHO, 140 million children were born every year and 5 million die in the first month of life in developing countries due to genetic preventable disorders. 4% of the population in India is mentally retarded and 5-15 % of sick new born have a metabolic problem³.

The screening of metabolic disorders is still a young science. Prof. Robert Guthrie Conceived the Concept in 1960 in USA and the first metabolic disorder tested was Phenylketonuria⁴. In India the first new born screening as a pilot screening project was carried out in 1980 in Bangalore, Karnataka for amino acid disorders involving 125,000 new borns⁵ screening the high risk populations, homocystenemia, hyperglycinemia, maple syrup urine disease, phenylketonuria, hypothyroidism and G6PD deficiency were found to be the common causes of mental retardation. The first expanded new born screening programme was initiated in Hyderabad, A.P. to screen all the new born. This study has shown a high prevalence of treatable inborn errors of metabolism. Interestingly a very high prevalence of inborn errors of metabolism to the extent of 1 in every 1000 new borns was observed⁶. Phenylketonuria the most common inborn error of amino acid metabolism is inherited as an autosomal recessive trait and is caused by impaired conversion of phenylalanine to tyrosine⁷. Although the overall incidence is less (1 in 12,000 live births) a few studies have shown high incidence (7 cases in 451) in mentally retarded patients⁸.

As the main objective of screening is early detection and early intervention to prevent disability and death, the present study of screening of metabolic disorders i.e., aminoacidurias was undertaken in children showing the signs and symptoms of aminoacidurias like mental retardation, seizures, failure to thrive, delayed milestones, speech defects, degenerative arthritis, cartilage pigmentation etc.

MATERIALS AND METHODS

The present study was carried out in the Dept. of Biochemistry, Sri Venkateswara Medical college, Tirupati. A total no. of 604 children aged below 5 years who presented with persistent vomiting, failure to thrive, unexplained mental retardation, developmental delay, motor deficits or convulsions, unusual odor, particularly during an acute illness, hepatomegaly, renal stones, speech deficits, microcephaly etc. and also children of parents having history of unexplained previous neonatal death and history of consanguinity were screened, from those attending the Pediatric O.P., and from the M.R. Board, Dept of Psychiatry S.V.R.R.G.G.H. Tirupati. Informed written consent was taken from parents of each child included in the study.

To diagnose accurately an inherited aminoaciduria in a sick infant or child certain precautions^{9,10,11} like, sample collection 48 hrs after birth, instructing breast feeding mothers to stop the drugs interfering with the urine amino acid assessment, collection of unhemolysed blood and mid stream urine sample without any preservatives were taken. 20 ml of random urine sample was collected into a sterile collecting cup, subjected to centrifugation at 5000 rpm for 15 min. and supernatant was used for performing the screening tests^{10,12,13}. Ninhydrin test for generalized aminoaciduria, Ferric chloride test for PKU/histidinemia/alkaptonuria, Dinitrophenyl Hydrazine test for PKU/MSUD/histidinemia, Benedict's test for alkaptonuria Silver Nitrate test for alkaptonuria and Cyanide – Nitroprusside test for sulphur containing aminoacids. 2 ml of heparinized blood samples (0.2 mg/ml) were collected aseptically from children showing positive

urine tests, plasma was separated by centrifugation, then urine and plasma was subjected to TLC⁶ and further confirmation was done by plasma reverse-phase HPLC^{6,16}.

RESULTS

In our study out of the 604 children who were screened by the urine screening tests, 3 children showed positive reactions as represented in table-1.

Table-1: Urine Screening Tests

S.No	Tests	Observation	Inference
1	Ninhydrin test	Purple colour is observed	Amino acids are present in urine
2	Ferric chloride test	Green colour was observed for 2 min.	May be phenyl pyruvic acid.
3	Dinitro phenylhydrazine test	An yellow ppt. was observed	May be PKU or MSUD
4	Benedict's test	No black ppt. was seen	Absence of alkaptonuria
5	Silver nitrate test	No silver deposit was observed	Absence of alkaptonuria
6	Cyanide nitroprusside test	No cherry red colour was seen	Absence of sulphur containing aminoacids

The urine samples of the 3 children which were positive for ninhydrin test, ferric chloride test and dinitro phenylhydrazine test were subjected to TLC, along with their plasma for qualitative assessment. Both urine and plasma samples showed prominent band on the TLC sheet which was corresponding to the phenylalanine standard as shown in figs 1&2.

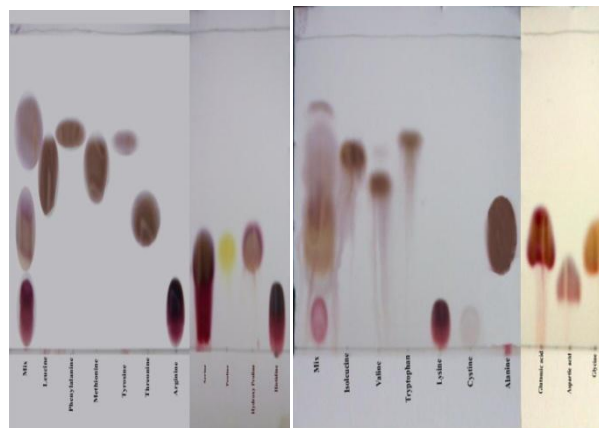
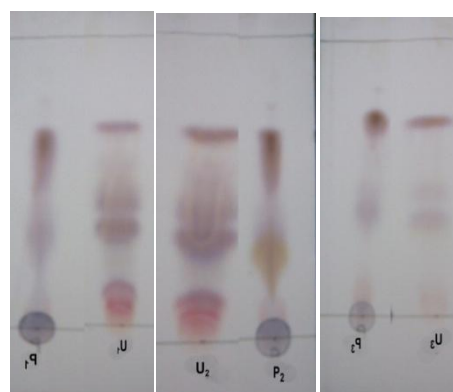


Fig. 1 TLC of Amino acid Standards



Patient 1 Patient 2 Patient 3
U-Urine, P-Plasma

Fig. 2 TLC of urine & plasma of patients

The R_f values of the spots produced by amino acids in the urine and plasma samples were compared with the reference standard amino acids and was identified as phenylalanine as represented in table-2. The plasma phenylalanine levels of the 3 children by HPLC were 12.1 mg/dl, 13.9 mg/dl, and 10.4 mg/dl respectively as shown in Fig: 3.in contrast to the normal levels of 1-3mg/dl.

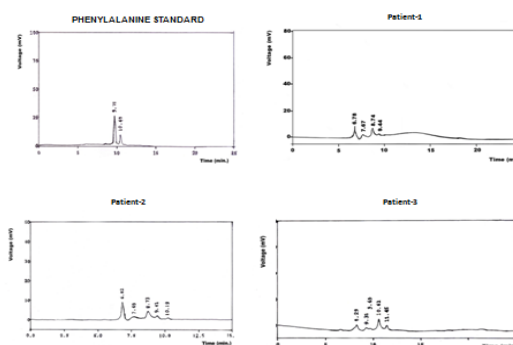


Fig: 3-HPLC of Phenylalanine std. & Patients

Table-2: R_f Values of Phenylalanine Std and amino acids in urine & plasma samples by TLC

S.no.	Phenylalanine std.	Patient 1		Patient 2		Patient 3	
		Urine	Plasma	Urine	Plasma	Urine	Plasma
1	0.65	0.66	0.65	0.65	0.64	0.65	0.65

DISCUSSION

A number of genetically based biochemical disorders related to amino acid metabolism were detected in infants and children by simple urine screening tests. Aminoaciduria is defined as increased amount of one or more amino acids in urine¹⁷. Though uncommon, it is more likely that one or more patients suffering from any of the aminoacidurias can be encountered by physicians or pediatricians or neurologists. So the detection of the disease in early life followed by appropriate treatment, genetic counseling can lead to a significant reduction in the infant mortality and reduces the number of mentally retarded children in the population.

Urine screening tests conducted on a routine basis on neonates, infants or children of any age, who fail to thrive, showing delayed milestones, mental retardation and neurological features will be of benefit. Mainly the mentally retarded children will be benefited by these urine screening tests for accurate diagnosis and timely intervention. Urine screening tests, nutritional guidance along with genetic counselling will be of value to prevent neurological deficit in subsequent siblings. Morbidity and mortality of phenylketonuria is still a bane on the society despite the fact that it can be diagnosed and treated at an early age.

The Guthrie's blood phenylalanine assay developed in 1961¹⁴ is still useful as a screening method, though it is time consuming. In the United Kingdom phenylketonuria is assessed in different Laboratories by Guthrie's method, TLC or fluorometry. In Turkey, Guthrie's test is still used as the sole screening method, whereas in France, Soviet Union and Hanover in Germany it is replaced by fluorometry¹⁵. From this it is apparent that there is a lack of consensus on the ideal methods for evaluating phenylketonuria.

In our study a combination of urine screening tests, urine and plasma TLC and confirmatory plasma phenylalanine assay by HPLC was done for diagnosis of phenylketonuria. Out of 604 children screened by urine tests, only 3 aged 2 yrs, 2 ½ yrs and 4 yrs respectively were found to be positive for aminoacidurias. The urine and plasma samples of these 3 children when subjected to TLC showed a typical chromatogram of phenylketonuria and this was confirmed by HPLC which showed increased plasma phenylalanine levels of 12.1 mg/dl, 13.9 mg/dl and 10.4 mg/dl respectively. Our observations of TLC and HPLC are co-relating with the results documented by other studies^{8,16,17,18}.

Early detection of aminoacidurias and effective treatment will prevent mental defect, or where in no

treatment is available, parental counselling can be given to prevent further siblings getting affected. Urine screening tests and TLC does not require special instrumentation¹⁷ and patients showing phenylketonuria spot in TLC can be referred to higher institutes for confirmation by HPLC.

Thus from our study it can be concluded that for a developing country like India mental retardation being one of the chief symptoms of phenylketonuria, evaluation by urine screening tests followed by TLC and confirmation of diagnosis by HPLC is practical and inexpensive .

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