Original Research Article

Biochemical screening tests in the urine of mentally retarded children

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ABSTRACT

Background: Inborn errors of metabolism (IEMs) are a group of genetically determined disorders caused by mutant genes that in turn produce abnormal enzyme to affect metabolism of a nutrient and a significant association has been found between mental retardation and IEMs. The present study was conducted on mentally retarded children to diagnose IEM using a panel of simple biochemical tests to be confirmed by various chromatographic techniques.

Methods: The study was done on 300 mentally retarded children admitted for treatment in the Paediatric Department, Govt R. M. Hospital, Thanjavur. Complete clinical history was collected in a predesigned proforma. 50 ml of urine samples was collected from each patient in a clean sterilized bottle and analysed for a series of biochemical tests using a standard protocol. All the observations were collected and analysed.

Results: Male dominance was seen in the study (65.3%). Out of 300, 93 children (31%) of the total were with the family history of consanguinity and the of rest 201 (69%) cases of non-consanguinity, delayed labour was found to be more common (50%). 4 children were affected with metabolic disorders (1.33%) i.e. 1 case was with phenyl ketonuria and other 3 cases with mucopolysaccharidoses.

Conclusions: The study identified four cases of IEM in the mentally – retarded children with underlying molecular defects and also paved a path to save the children in the case of phenylketonuria by the treatment i.e. diet low in phenyl alanine.

Keywords: Biochemical screening tests, Mentally retarded children, Urine samples

INTRODUCTION

Inborn errors arises because of a particular enzyme catalysing a single metabolic step in a series of step-wise reactions occurring in specific metabolism, is reduced in activity or missing altogether, so that the substrate of the enzyme as well as other substances derived from the substrate by other routes accumulate in the body and are eventually excreted in the urine.¹⁻³

In other words, a defective enzyme causes an impairment in the metabolism of a nutrient and the fault in the enzyme is largely a result of imperfect gene. The one gene – one enzyme concept has an immediate explanatory potential for inborn errors of metabolism

(IEM). It is to say that inborn errors of metabolism are caused by mutant genes that in turn produce abnormal enzyme to affect metabolism.

Thus, mutation becomes the origin of genetic diseases and a significant association has been found between mental retardation and IEMs.⁴⁻⁶ The mechanisms of inborn errors and the modes of their detection came from studies of urine and blood.⁷

The exact site of metabolic derangement is determined from a direct assay for activity of the suspect enzyme in the blood or tissue obtained by biopsy. The urinary excretion of excessive substrate, or deficient product of the missing enzyme can be identified by chemical

methods, although several inherited disorders are associated with specific unusual odour, unusual urinary

colour or with urinary crystals for easy identification of them (Table 1).⁸⁻¹⁶

Table 1: Screening tests for the presence of specific metabolites in urine.

Name of the test	Substance showing positive tests	Disease
Ferric chloride test	Imidazole pyruvic acid, Imidazole lactic acid	Histidinemia
	Alpha keto- isovaleric acid, isocaproic acid, beta methyl valeric acid	Maple syrup urine disease
Nitroso naphthol test	Tyrosyl group containing compounds like tyrosine and its metabolites	Tyrosinemia
Nitroprusside test	Sulphydryl group (-SH) and disulphide group (-S-S-) containing compounds	Homocystinuria, Cystinuria
Ninhydrin test	Alpha amino acids	Amino aciduria
Cetyl trimethyl ammonium bromide (CTAB) test	Mucopolysaccharides	Mucopolysaccharidoses type 1 and also connective tissue disorders with less marked reaction
Berry test	Mucopolysaccharides	MPS
Toluidine blue staining for metachromatic granules	Metachromatic granules	Metachromatic leukodystrophy and rarely tay-Sach's disease
Benedict's test	Reducing sugars, glucuronate, salicyluric acid, homogentisic acid, Vit C etc.	Galactosemia, fructosuria
Dinitro phenyl hydrazine test	Alpha- keto acids	Maple syrup urine disease, Phenyl ketonuria, Histidinemia, Tyrosinemia

Most colour tests are non-specific and hence positive responses in the tests are confirmed further by chromatograms.⁸⁻¹¹ These screening tests, that are simple, reliable, reproducible, inexpensive and suitable for developing country like India, are useful in the detection of relatively more common metabolic disorders such as phenyl ketonuria, histidinemia, galactosemia, Hartnup disease, maple syrup urine disease, homocystinuria, mucopolysaccharidoses and metachromatic leukodystrophy.¹²⁻¹⁶ They can be applied on a large population to be screened for specific metabolic disorders, thereby facilitating the detection of more number of affected subjects, prior to the onset of clinical problems. The factors like age, concentration of the urine, intake of drugs, type of food may interfere in these tests, thereby giving false results. These pitfalls are eliminated by carrying out chromatograms simultaneously.

The present study was done with the aim 1) to identify the children mentally affected due to inborn errors of metabolism, among mentally retarded children, 2) to find out the possibility of treating the conditions, wherever possible, so as to allow the children not only to survive but also to lead an almost normal life, 3) to decide whether metabolic abnormality is the leading cause for mental retardation in children, 4) to assess the prevalence of the disorders among children born of parents closely related to each other i.e., due to consanguinity, 5) to evaluate whether sex has any role to play in the incidence

of the disorders and 6) also to explore the credibility of screening tests in identifying selected metabolic diseases.

METHODS

This descriptive diagnostic study was conducted from April 2000 to March 2002 on 300 mentally retarded children admitted for treatment in the Paediatric Department, Govt R. M. Hospital, Thanjavur after getting approval from Institutional ethical committee. Informed consent was taken from the parents/guardian of the child. All were subjected to selective screening tests. A detailed clinical history was obtained from the parents in the pre designed proforma. A through physical examination was also carried out.

Selection criteria

Inclusion criteria

Mentally retarded child between ages 5 to 12.

Exclusion criteria

Mentally retarded children with radiologically proven brain tumour, infection, trauma.

50 ml of non-fasting urine was collected from each patient in a clean sterilized bottle. Patients were instructed to stop all the drugs and synthetic foods for 3

days before taking the sample.^{8,9} Since the tests were done immediately after collection, no preservative was used.

A set of simple and cost effective biochemical screening tests were performed for identification of urinary reducing substances (carbohydrates), amino acids, keto acids, mucopolysaccharides, copper, ketone bodies, porphyrins and other metabolites (Table 1). Confirmatory tests like thin layer and paper chromatography for identification of sugars and one dimensional paper chromatography for identification of amino acids was also done.⁸⁻¹¹

All the data were collected, documented in excel, analysed and presented in number and percentages.

RESULTS

Urine samples collected from all the patients were subjected to biochemical tests as mentioned in Table 1. Among the cases screened male preponderance was seen (65.3%) than females (34.7%). Of the 300 mentally retarded children selected for screening, 93 children (31%) of the total were with the family history of consanguinity and the rest 201 (69%) were born of parents unrelated. Figure 1 revealed the percentage of mental retardation due to various causes other than metabolic disorders. Delayed labour was found to be more common (50%), giving rise to formidable deterioration to the brain function followed by history of abortion (14%).

Table 2: Patients characteristics (n=300).

Characteristics	Number (%)
Sex	
Male	196 (65.3)
Female	104 (34.7)
Family history of consanguinity	
Present	93 (31)
Absent	207 (69)

Table 3: Number of positive tests and real positive cases confirmed by chromatogram.

Name of the test	No. of cases	Number of real positive cases confirmed by chromatogram or CTAB
Ninhydrin test	19	0
Ferric chloride test	1	1
Nitroso naphthol test	9	0
Cyanide nitroprusside test	6	0
DNPH test	1	1
Benedict's test	3	3 (Muco)
Berry test	3	3
Toluidine test for metachromic granules	0	0

The results of biochemical screening tests were given in Table 3. The maximum numbers of positive reaction were obtained with Ninhydrin test, a well-known colour reaction for alpha-amino acids but none of them was due to the presence of amino acids, as judged from chromatogram. Cyanide nitroprusside test for -SH (or) S-S-group was positive in 6 cases but unable to confirm the existence of either cystinuria or homocystinuria in all these patients by urinary chromatogram. DNPH test, devised exclusively for the identification of alpha keto acids, was positive in only one case of phenyl ketonuria. Three cases were positive with Benedict's test. None of them was found to contain simple sugar, as shown by chromatograms. All 3 cases were recognised as MPS by Berry test, which was confirmed by CTAB test.

Figure 1: Mental retardation for reasons other than metabolic errors.

In the present study, out of 300 children, 4 children were affected with metabolic disorders (1.33%), 2 were males (1.03%) and the remaining 2 were females (1.9%). Of them, 1 case was with phenyl ketonuria and other 3 cases with mucopolysaccharidoses. The numbers affected with mental retardation due to disorder in the case of consanguinity was 2 and that in non-consanguinity was 2.

Table 4: Children diagnosed with metabolic disorders by means sex, family history and disease (n=300).

Children diagnosed with metabolic disorders	N (%)
Number of children affected with metabolic disorders	4 (1.33)
a. By sex	
Males	2 (1.03)
Females	2 (1.9)
b. By family history of consanguinity	
Present	2 (0.66)
Absent	2 (0.66)
c. By disease	
Phenyl ketonuria	1 (0.33)
Mucopolysaccharidoses	3 (1.00)

DISCUSSION

The object of the present study was three fold. It was felt essential and useful to identify and isolate children afflicted with inborn errors of metabolism within a group of mentally retarded children and to find out the possibility of treating the children by all possible means so as to allow them to lead essentially normal life, apart from giving genetic counselling and helping the particular parents regarding the future normal progeny. Towards achieving the object, the programme was devised. In order to materialize the programme, three hundred mentally retarded children were selected for screening tests from those stepped into R. M. Hospital, Thanjavur for treatment during the past two years.

The findings of the present study revealed that there were more males with mental retardation than females. This was in agreement with the findings of Jaiikhani et al.¹⁷ The sex – based statistics in this study, revealed that there were 1.03% males with metabolic defects among the 196 males, against 1.9% females with defective metabolism among the 104 females, suggesting that metabolic derangements were more common in females. On contrary to this, Phornphutkul et al, however, reported a view with respect to alkaptonuria, an inborn error of tyrosine metabolism. According to them, alkaptonuria is relatively more common in males.¹⁸

In the present study, the numbers affected with mental retardation due to disorder in the case of consanguinity was 2 and that in non-consanguinity was 2. The genetic disadvantages of a consanguine marriage are universally accepted but the precise risk remains to be established. The fact that equal numbers, compared to non-consanguinity, are affected with mental retardation in the case of consanguinity, clearly shows that the risk is considerable in children, born of parents married among close relatives, i.e. in children with family history of consanguinity. Yadar et al reported that in a quantitative amino acid analysis in 800 subjects over a three year period in Al-Sabah hospital, Kuwait, thirty five percent were seen with amino acidopathy and two of them were from first degree of consanguineous marriage.¹⁹

There is evidence to show that, although consanguinity is one of the reasons for mental derangement, a variety of other causes act as important factors in the production of mental retardation.²⁰ In this study, family history of mental sub-normalities among parents and other close relatives were presents in 6% of cases. The various abortifacients taken by the mother to terminate pregnancy were found to be a reason for mental retardation in some children. Delayed labour was found to be major cause of mental retardation (50%) in children in the present study. Other reasons included are head injury, maternal and paternal diabetes, maternal malnutrition, thyroid deficiency etc. The above facts suggest that there are various factors, other than consanguinity, that play crucial roles in the incidence of mental retardation.

In the present study, 1 case of phenyl ketonuria (PKU) and 3 cases of mucopolysaccharidoses were identified, indicating a total number of 4 positive cases out of 300 cases screened or 1.33% positive cases.

The classical PKU, an autosomal recessive disorder of phenyl alanine metabolism, is caused by deficient activity of phenyl alanine hydroxylase with the accumulation of phenylpyruvic acid in the blood and tissue and excretion of phenyl pyruvic acid along with other metabolites such as phenyl acetic and phenyl lactic acid in urine. In classical PKU, the mousy odour of urine, due to phenylacetic acid, may occur early in the first year of life but it may not be a complaint of the parents. The child will, however, loose 50% I.Q. point in that year. After the first year, eczema, delayed psycho motor developments, failure to walk and talk, as well as seizures are found. The lack of symptoms in the first year and a considerable loss of I.Q. in the same year are one of the compelling reasons for screening for PKU in neonatal period.²¹

Dietary restriction of phenyl alanine intake is the only practical therapy for PKU at present. The earlier the treatment, the better will be the cure. The requirement of phenyl alanine varies with age, from 47- 90mg/Kg/day during 2-4 months of age and approximately 27mg/Kg/day after one year of age.²² The requirement also varies from one child to another, depending on the amount of enzyme activity. However, maintaining the blood phenyl alanine level below normal has resulted with poor growth, retarded bone age, hepatomegaly, repeated infection, hypoglycaemia and neurological symptoms. Blood phenyl alanine is measured weekly once for the first year but once in every 4 weeks thereafter. The restricted diet can be discontinued at 8 to 10 years of age.

Three children with features of mucopolysaccharidoses were identified in this study. Of which two children were with features of Hurler syndrome and remaining one was with features of Hunter syndrome. Their urine samples showed marked flocculation with CTAB reagent and typical violet ring was seen with Berry test.

Mucopolysaccharidoses are a group of lysosomal storage disorders. Hurler disease, an autosomal recessive disorder, may be considered as the prototype for the other mucopolysaccharidoses. In this disorder, the defective enzyme is L iduronidase. The enzyme defect leads to accumulation of heparin sulphate and dermatan sulphate within the lysosomes of various tissues and organs of the body. Clinically, the skeletal changes and intellectual impairment are severe. The striking features are large, long head, short, broad hands, stubby fingers, joint stiffness, clouding of cornea and mental retardation after the first year. Death frequently results during the first decade. Behaviour problems are a primary feature of the mucopolysaccharidoses and play a major strain on families.²³

No specific treatment is available at present. In fusion of normal plasma as a possible means of replacing the missing enzyme has improved transiently in some patients, although the level of circulating L iduronidase is low. Leukocyte transfusion and fibroblast transplantation have resulted in quantitative changes in MPS excretion but without clinical amelioration.²³

In general, the clinical features of the Hunter syndrome are similar to those of the Hurler syndrome, but somatic changes and mental retardation may be less severe. Progressive deafness occurs at an early age. Death frequently results from cardiac causes in the first decade, but some patients may live into the third or fourth decade. The X- linked mode of inheritance of the Hunter syndrome assists in its differentiation from the Hurler syndrome. Deficient enzyme in Hunter syndrome is L-iduronosulphate sulphatase. The accumulated metabolites are heparin sulphate and dermatan sulphate.²⁴

There are ways and means to prevent the birth of abnormal children and to escape from the hazardous situation. Genetic counselling is an apt method to avert the condition by avoiding the marriage of two heterozygotes for the same defective gene being got married. Even if the two are already married without knowing the fact of being heterozygote, there is a possibility of preventing the birth of affected infant, if the disease can be diagnosed in vitro by aminocentesis. The use of transabdominal aminocentesis permits diagnosis of certain genetic diseases at a stage early enough to terminate pregnancy and to prevent the birth of defective child. This method gives the opportunity to have unaffected children, provided the parents are willing to terminate the pregnancy in the event that an abnormal foetus is detected.²⁵

Another promising antenatal diagnosis involves a knowledge of the anatomy of the human genome. Prenatal diagnosis and carrier detection are possible by using specific genetic probes in cells obtained from chorionic villus biopsy. If molecular genetic facilities are not available, diagnosis can be made by phenyl alanine hydroxylase level in foetal liver. Since the enzyme is absent in amniotic fluid and blood, foetal liver biopsy is necessary to estimate the enzyme level.

Regarding the treatment aspect of inborn errors, in the recent years, many methods have been devised and carried out with little or greater success at least in some cases. There are diversity of methods, as are the variety of metabolic defects. Some of them are simple. The simplest method of treatment consists of diet restriction. But the availability of food product devoid of particular substance is considered to be the most difficult part of the method. The other methods adopted to cure the metabolic defects are somewhat cumbersome in nature and in the list are included organ transplantation, surgical removal of certain organs, genetic counselling and gene therapy.²⁶

The present study helped not only to identify four cases of metabolically oriented, mentally – retarded children with underlying molecular defects but also paved a path to save the children from disastrous end, at least in the case of phenylketonuria by the treatment given to the child i.e. diet low in phenyl alanine.

CONCLUSION

Metabolic disorders are an important cause of death in the first decade of life in children. Genetic factors individually or in combination with exogenous factors, form the basis for metabolic defects. It is therefore necessary to identify gene– related metabolic defects and can be achieved by mass screening programme. The old conventional methods of mass screening are not only tedious and cumbersome but also elaborate and expensive. The simple and inexpensive screening tests adopted in the present study can be used as an alternate device to achieve the same purpose and a major advantage in performing these tests is that they are done in the urine, thereby avoiding the difficulties in the collection of specimens.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Bearn AG, Miller ED. Archibald carried and the development of the concept of inborn errors of Metabolism. *Bull Hist Med.* 1979;53:315.
2. Ladubn Zannoni VA, Laster L, Seeg Miller JE. The nature of the defect in tyrosine metabolism in Alcaptonuria. *J Biol chem.* 1958;230:251.
3. Sutton HE, Wagner RP. Mutation and enzyme function humans. *Ann Rev Genet.* 1975;9:187.
4. Harris H. The principles of human biochemical genetics. Ed.3 Amsterdon, North Holand: Publishing Co;1980:12.
5. Datar CA, Apte BN. Screening of previously undiagnosed pediatric cases of mental retardation and autism for specific metabolic disorders. *Bombay Hospital J.* 2008;50(4):560-5.
6. Gupta A. To err is Genetics: diagnosis and management of inborn errors of metabolism (IEM). Chapter 32. 415-423.
7. Bondy, Rosenberg. *Metabolic control and disease.* 8th Ed. 1981: 91.
8. Smith I. *Chromatographic and Electrophoretic Techniques.* Volume 1. Chapter 1. William Heinemann Medical Books, London: Interscience Publishers; 1976.
9. Smith I. *Chromatographic and Electrophoretic Techniques.* Volume 1. Chapter 5. William Heinemann Medical Books, London: Interscience Publishers; 1976.

10. Henry RJ. Clinical chemistry. Principles and techniques. Chapter 13. 1964.
11. Smith I. Chromatographic and Electrophoretic Techniques. Volume 1. Chapter 11. William Heinemann Medical Books, London: Interscience Publishers; 1976.
12. Stuber A. screening test and chromatography for the detection of inborn error of metabolism. *Clin Chimica Acta*. 1972;36:309.
13. Stanbury JB, Wyngaarden JB, Fredrickson DS. The Metabolic Basis of Inherited Disease. 3rd edition. New York: McGraw-Hill Book Co; 1972: 275.
14. La Du BN, Howell RR, Jacoby GA, Seegmiller JE, Sober EK, Zannoni VG, et al. Clinical and biochemical studies in two cases of histidinemia. *Pediatrics*. 1963;34:216.
15. Auerbach VH, DiGeorge AM, Baldrige RC, Tourtellotte CD, Brigham MP. Histidinemia: a deficiency in histidase resulting in the urinary excretion of histidine and of imidazolepyruvic acid. *J Pediatr*. 1962;60:487-97.
16. Pennock A. A review and selection of simple laboratory methods used for the study of glycosaminoglycin excretion and the diagnosis of mucopolysaccharidosis. *J Clin Path*. 1976;29:111.
17. Jaikhani R, Patil VS, Laxman HB, Shivashankara AR, Kulkarni SP, Ravindra MS. Selective Screening For Inborn Errors Of Metabolism In Children: Single Centre Experience From Karnataka. *J Clin Diagnostic Res*. 2008;2:952-8.
18. Phornphutkul C, Introne WJ, Perry MB, Bernardini I, Murphey MD, Fitzpatrick DL et al. Natural history of alkaptonuria. *N Engl J Med*. 2002;347:2111-21.
19. Yadav GC, Reavey PC. Aminoacidopathies: a review of 3 years experience of investigations in a Kuwait hospital. *J Inher Metab Dis*. 1988;11(3):277-84.
20. Milunsky A. The prevention of genetic disease and mental retardation. Philadelphia: WB Saunders; 1975; 38-39.
21. Targum SD, Lang W. Neurobehavioral problems associated with phenylketonuria. *Psychiatry (Edgmont)*. 2010;7(12):29-32.
22. Phenylketonuria (PKU). Available at: <https://www.healthline.com/health/phenylketonuria>. Accessed on 10 February 2018.
23. Martins AM, Dualibi AP, Norato D, Takata ET, Santos ES, Valadares ER, et al. Guidelines for the Management of Mucopolysaccharidosis Type I. *J Pediatr*. 2009;155(4):32-46.
24. Lin H, Chuang C, Chen M, Lin S, Chiu P, Niu D, et al. Natural History and Clinical Characteristics of Taiwanese Patients with Mucopolysaccharidosis II: Data from the Hunter Outcome Survey (HOS). *J Inborn Err Metabolism Screening*. 2016;4:1-82.
25. Dundar M, Uzak AS, Erdogan M, Akbarova Y. Prediction, prevention and personalisation of medication for the prenatal period: genetic prenatal tests for both rare and common diseases. *EPMA J*. 2011;2(2):181-95.
26. Chung W. Neuromuscular disorders of infancy, childhood, and adolescence (Second Edition) A Clinician's Approach. In: Darras BT, Royden Jones H, Ryan MM, De Vivo DC, editors. Elsevier; 2015: 17-31.

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