DETECTION OF INHERITED METABOLIC DISEASES IN CHILDREN WITH MENTAL HANDICAP

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ABSTRACT

Mental Retardation is a condition where complex interactions of intrinsic and extrinsic factors hamper mental and sometimes physical growth of the child during developmental period. This study was carried out to detect cause of Mental Retardation in 2000 cases of developmental delay by a multidisciplinary team comprising of a Pediatrician, Cytogeneticist, Biochemist, Psychologist and Speech and Occupational therapists. The causes for developmental delay are broadly divided into Genetic, Environmental and Idiopathic (no specific cause found) factors. The complete diagnosis was possible in 1192(60%) cases. Genetic factors were found in 477(23.8%) cases and environmental factors covered 692(34.6%) cases as a cause of retardation. The most common genetic cause is chromosomal abnormalities which were found in 355 (17.75%) cases. Another major group comprising of 122 (6.1%) cases was that of the disorders which follow Mendelian inheritance. Autosomal recessive conditions, which covers majority of metabolic disorders, are detected in 48(2.4%) cases. During this screening the most common inherited metabolic condition detected is Mucopolysaccharidosis, a Lysosomal Storage Disorder followed by Amino acid abnormalities like Phenylketonuria, Alkaptonuria and Tyrosinuria.

KEY WORDS

Mental Retardation, Inborn Errors of Metabolism, Autosomal Recessive Inheritance, Genetic Counselling.

INTRODUCTION

The American Association on Mental Retardation (AAMR) revised the definition of mental retardation (1992) and it refers to significantly sub average intellectual functioning (defined as an I.Q. score of below 70) existing concurrently with limitation in two or more of the following adaptive areas like communication, self care, home living, social skills, self direction, health and safety, leisure, work and functional academics (1). The severity of mental retardation has been based on the IQ score, the various groups as per the WHO classification are – Borderline (IQ, 70-40); Mild (IQ 55-69); Moderate (IQ 40-54); Severe (IQ 25-39) and Profound (IQ below 25). Mental Retardation is a condition with varying etiology. It may have genetic or non-genetic (environmental)

origin. Genetic causes of mental retardation are broadly classified into Single gene disorders including all the inborn errors of metabolism, Chromosomal disorders and those which are multifactorial in origin. Mental Retardation can be due to static or progressive encephalopathy (2). Progressive encephalopathy is where the child loses already acquired milestones and regresses from the higher level of CNS functioning to a lower level. It is due to neurodegenerative disorders which are very often metabolic in origin.

Inborn Errors of Metabolism (IEM) can be suspected in an infant or child with one or more of the following manifestations.

- Progressive disease with intermittent or continuous loss of skills
- Convulsions, regression in neurological status.
- Unusual odor, particularly during an acute illness
- Intermittent episodes of unexplained vomiting, acidosis, mental deterioration or coma.
- Characteristic signs e.g. Hepatosplenomegaly, coarse faces, abnormal hair color, texture etc.
- Consanguinity, family history of mental retardation.
- Sudden Infant Death Syndrome (SIDS)

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Early diagnosis is very important since the condition is rapidly progressive and results into irreversible damage in early childhood. It also helps in initiating the treatment in the earlier stages of development as it will be more effective and will improve the outcome at a later stage. The most important aspect of early diagnosis is that, it helps in genetic counselling. In a hospital based study in India biochemical screening of 4400 cases of mental retardation revealed that 5.57%(256 cases) were due to metabolic disorders (3). One of the studies shows estimated incidence of metabolic disorders in the range of 3-4 per thousand new born babies (4). Although the frequency of each inborn error of metabolism is low, overall incidence of inherited metabolic disease is high. Hence it becomes mandatory to carry out biochemical investigations for detection of inherited metabolic disease in children with mental handicap.

MATERIALS AND METHODS

In the present study 2000 cases with chief complain of developmental delay referred by Psychiatrists, Pediatricians and other professionals working in this field and from Special Schools were investigated. Child with Mental Handicap was examined by a Pediatrician and Psychological assessment (IQ) was done by clinical Psychologists using method modified for Indian Children by Kamath(5), and social quotient by Vineland Social Maturity Scale modified for Indian Children by Mallin(6). Detection of Inborn Errors of Metabolism was carried out using following techniques.

Table I : Standard Screening Tests to detect Inborn Errors of Metabolism Associated with Mental Retardation

Name of the Test	Disorders or compound detected
Ferric Chloride	PKU, Tyrosinemia, MSUD, Histidinemia, Alkaptonuria, Acetoacetic Acid, Salicylates, p-Aminosalicylic acid, Phenithiazine, Antipyrine, Isoniazide.
Dinitrophenyl Hydrazine Test(DNPH)	MSUD, PKU
Nitrosonaphthol Test	Tyrosine metabolites
Cyanide- Nitroprusside Test	Cystine, Homocystine in Homocystinuria
Silver Nitroprusside Test	Homocystinuria
Acid Albumin Turbidity Test/ Alcian Blue Spot Test	Mucopolysaccharidosis
Benedicts Qualitative Test	Reducing Sugars

Urine metabolic Screening: These screening tests are carried out to detect wide range of metabolic abnormalities of amino acids, Carbohydrates, some of the Organic acids and Mucopolysaccharidosis(7,8). The details of the various screening tests are given in Table-I

Plasma and Urinary amino acids detection by Paper Chromatography: 1-Dimensional paper chromatography for plasma and urinary amino acids with the solvent system comprising of 1- Butanol: Glacial Acetic Acid: Water (12:3:5) and developing with Ninhydrin (0.5% in Acetone) covers detection of most of the inborn errors of amino acid metabolism (9). The amount of urine to be loaded depends on the Creatinine concentration in mg/100ml. Confirmation of Amino Acids which are detected during 1-Dimensional Paper Chromatography in plasma and urine is done by Thin Layer Chromatography with respective standard reference amino acids using solvent system 1-Butanol: Acetone: Acetic Acid: Water (6:6:3:5) and development with Ninhydrin(9).

Thin layer chromatography(TLC) for Sugars/ Oligosaccharides: This diagnostic procedure involved TLC with standard sugar solutions of Lactose, Sucrose, Galactose, Glucose, fructose, Xylose(100 mg/100ml water with respect to each and 10mg. Merthiolate as preservative) with solvent system Ethyl Acetate: Pyridine: water (6:3:2) v/v in first development and second development in N- Butanol: Acetone: Acetic Acid: D/W(35:35:10:20) and developing with P- Amino Benzoic Acid reagent (700 mg in 0.4ml Orthophosphoric Acid and diluted to 100ml with Methanol) by dipping the chromatograms followed by drying and heating it at 100-115°C in the hot air oven. This technique is used to detect Galactosuria, Lactosuria, Pentosuria and others, Sialidoses, Fucosidosis or Mannosidosis (10).

Cellulose Acetate Electrophoresis: This technique is carried out on Cellulose Acetate Strip (2.5x15cm - Sartorius make) to detect various components of Mucopolysaccharides, if the initial screening test is positive for Mucopolysaccharidosis. The urine sample (20 ml) is incubated overnight with 1 ml Cetyl Pyridinium Chloride(10 gm% in Distilled Water) to precipitate Mucopolysaccharides and then centrifuged the precipitates are incubated over night with 1 gm% Potassium Acetate in Ethanol. The sample is centrifuged next day and the precipitates are dissolved in minimum amount of distilled water and spotted on Cellulose acetate strip which is saturated with Pyridine: Acetic Acid: Water(89:1:10) as solvent and run is carried out at 8 milliamp. for 30 minutes. The strip is dried and stained with 1 gm% Acian Blue prepared in Ethanol and Acetic Acid (Glacial).Excess dye is washed with water. The

Table II : Details of the Investigations and findings of cases with Inborn Errors of Lysosomal Enzymes

Proband	Clinical Features	Biochemical Investigations		Degree of M. R.	Diagnosis
		Screening Tests Alcian Blue Spot test/ Acid Albumin Turbidity test	MPS Electrophoresis	Specialized Tests Lysosomal Enzymes	
PS	CF, DM, HSM,SS, DD	Positive	EPP-HS	α - NAG levels <0.09 m μ /ml	Profound MPS-III
MC	CF, DM, HSM,SS, DD	Positive	DS+ HS	Not done	Severe MPS-VII
NZ	CF, DM, HSM, DD	Positive	DS+HS	Not done	Moderate MPS-VII
MH	CF, DM, HSM, DD	Positive	DS+HS	Not done	Profound MPS-
VS	CF, DM, HSM, DD	Positive	HS	α - NAG levels <0.09 m μ /ml	Profound MPS-III
KB	CF, DM, HSM, DD	Positive	HS	α - NAG levels 0.3mu/ml	Severe MPS-III A or C
AS	CF, DM, HSM, DD	Positive	DS+HS		Profound MPS-II(XR)
YK	CF, DM, HSM, DD	Positive	DS+HS	α - NAG levels 0.41mu/ml	Moderate MPS-II
PM	CF, DM, HSM, DD	Positive	DS+HS		Profound MPS-II(XR)
AK	CF, DM, HSM, DD	Positive		Not done	Severe MPS
MB	CF, DM, HSM, DD	Positive		Not done	Severe MPS
MP	CF, DM, HSM, DD	Positive		Not done	Moderate MPS

CF- Coarse Faces; DM- Disostosis Multiplex; HSM- Hepatosplenomegaly; DD-Developmental Delay; SS- Short Stature; HS- Heparan Sulphate; DS- Dermatan Sulphate; CS- Chondroitin Sulphate; MPS- Mucopolysaccharidosis

CPC precipitated Glycosaminoglycans are also differentiated using 0.1M Barium Acetate as buffer pH 8 and carrying out electrophoresis at constant current 1mA/cm for 4 hours. In both the above mentioned buffer system Chondroitin-6 Sulphate runs ahead of all the components followed by Dermatan Sulphate and then Heparan Sulphate. Detection of Keratan Sulphate was not possible by using these methods. After confirmation of the clinical features, positive screening tests (Acid Albumin Turbidity test and Alcian Blue Spot test) and identification of the components by Cellulose Acetate electrophoresis, the cases were referred to other centers for Lysosomal Enzyme studies.

For all the cases investigated at our center by Pediatrician, if specialized investigations like CT Scan, MRI, and EEG were required they were referred to other centers for the same and diagnosis was done after following up with these reports.

RESULTS AND DISCUSSION

In the span of seven years 2000 cases from wide referral network (Fig I) have been investigated. The complete diagnosis was possible in 1192 (60%) of cases (Fig II). Diagnosis of the causative factor of Mental Retardation helps

in the management of the affected child. In our study the genetic causes of mental retardation cover 477 cases (23.8%) and environmental causes cover 692(34.6%). In the category of genetic causes the chromosomal anomalies are the most frequent with 355 cases (17.7%) and Mendelian disorders

Figure I : Percentage wise break up of various referral sources from where patients were referred to our centre showing maximum referrals from medical faculty comprising of Psychiatrist, Neurologist, and Pediatricians etc.

Figure-II: Break up of Causes of Mental Retardation.

covered 122 cases (6.1%) with metabolic abnormalities in 26(1.3%) cases. According to one recent study screening of cases of mental retardation for amino acid disorders revealed four to be the commonest—Hyperglycinemia, Homocystinuria, Alkaptonuria, and maple syrup urine disease (MSUD). Metabolic studies of cases of mental retardation in AIIMS, Delhi and KEM Hospital, Mumbai, demonstrated that common disorders were those of Mucopolysaccharides, Lysosomes, Wilson disease, glycogen storage disease and Galactosemia (11). Similar earlier study mentions 35% cases of mental retardation due to chromosomal anomalies, 20% with Identifiable Genetic Syndrome and 8% with metabolic abnormalities (12). Kusuma Kumari et al have reported 29.3% due to chromosomal anomalies (13). A multicentric study of ICMR revealed that 4.9% of genetic causes of mental retardation were due to metabolic disorders (14). The low percentage of genetic causes in our study could be due to our data covering limited number of cases with mental handicap and mostly in the age range of 3-12 years and we do not come across neonatal population with metabolic abnormalities like a hospital set up.

The Mucopolysaccharidosis (MPS) - In our study we have detected 12 cases of MPS. Details of the investigations are in

Figure III : Child with MPS-III showing Coarse faces, and Hepatosplenomegaly

Table II. The most common subtype detected is MPS-II and MPS-III followed by MPS-VII. The diagnosis of MPS-II and MPS-VII was also supported on the basis of progressive encephalopathy with neuroregression in these cases. There were 3 confirmed cases of MPS who were not differentiated into subtypes due to lack of follow up as parents of these cases were not interested in further investigations. (Child with clinical features of MPS is shown in figure-III and Cellulose Acetate Electrophoresis in Figure-IV. Alcian Blue Spot test is shown in Figure-V)

Table III : Families with more than one child affected with Inborn Errors of Metabolism Resulting in Mental Retardation

Proband	Disorder	Consanguinity in parents	Family History of Mental Retardation/Relation of the family member with proband
MC	MPS	I- cousins	Present/Sibling (sister)
NZ	MPS	I-Cousins	Present/Sibling (brother)
KB	MPS	Absent	Present/ distant paternal cousin
AS	MPS	Absent	Present/ Maternal cousin
PM	MPS	Absent	Present/ Sibling(brother)
VV	PKU	I- cousins	Present/ paternal cousin
GD	Xeroderma Pigmentosum	Dist. Cousins	Present/ Sibling(brother)

Figure-IV: Cellulose Acetate Electrophoresis Showing (1) Standard Dermatan Sulphate on the left(DS) and sample with Dermatan Sulphate and Heparan Sulphate and on the right Standard Heparan Sulphate(HS) with Pyridine: Acetic Acid:D/W buffer (2) Standard Chondroitin Sulphate(CS) and Dermatan Sulphate(DS) in 0.1M Barium Acetate buffer and (3) showing standard Dermatan and Heparan Sulphate DS and HS. with Pyridine : Acetic Acid: D/W as buffer system resp.

Metachromatic Leukodystrophy (MLD) is inherited disorder of Myelin metabolism. We have detected MLD in a child with chief complaints of inability to walk, speak and poor comprehension. Child had undergone CT and MRI scan showing diffuse leukoencephalopathy. Detection of enzyme levels of β - Galactosidase, and Aryl sulfatase were carried out in other centre and Aryl sulfatase activity was absent in proband. The very low or absent activity of Arylsulfatase A strengthened the diagnosis of metachromatic Leukodystrophy.

Inherited conditions associated with amino acid metabolism are the second most common metabolic errors. **Phenylketonuria (PKU)** was detected in a 3years 6 months old girl. The initial screening test showed positive Ferric chloride [Green colour of Phenyl Pyruvic Acid (PPA) and Phenyl Acetic Acid (PAA)] Dinitrophenylhydrazine test (Yellow Precipitates of PPA and PAA). Further confirmation was carried

out with 1-D paper chromatography (Semi Quantitative with varied concentration of standard Phenylalanine solutions comprising of 10mg/dl; 20 mg/dl; 30mg/dl of Phenylalanine, compared with plasma Phenylalanine levels) and thin layer chromatography of plasma and urinary amino acids (figure-VI). This showed elevated levels of Phenylalanine (above 30 mg/dl) in plasma. The child was referred to another centre for quantitative estimation of plasma Phenylalanine by HPLC and it was found to be 2678 μ M/gm Creatinine in urine and 894 μ M/L in plasma. The child was advised to have low Phenylalanine diet to bring down the amino acid levels in blood. After 8 months of dietary restriction of phenylalanine, plasma phenylalanine levels revealed marked reduction to 450 μ M/L and urine to 1756 μ M/gm Creatinine.

Ocuculocutaneous Albinism (OCA) in humans exists in 10 distinct forms. The child with Tyrosinase- Negative OCA came to us with chief complaints of developmental delay, strabismus and horizontal nystagmus with hypo pigmentation of eyes, hair and skin. The OCA phenotype is characterized by a complete absence of visible pigment. Hair bulb test was carried out by epilating the hair as described by Scott et al (15) and the root portion was immediately immersed in four different solutions

Figure-V (a) Alcian Blue Spot test showing (1) Negative Control, (2) Standard Chondroitin Sulphate, (3) Positive Control, (4) Negative sample.

Figure-V (b): Alcian Blue Spot test showing (1) & (3) Negative Control, (2) Negative sample. (4) Positive sample

Figure-V (c): Alcian Blue Spot test showing (1) Positive sample & (2, 3, 4) Negative Samples.

Figure-VI: Thin layer Ascending Chromatography of Urine and Plasma sample of child with Phenylketonuria. From L to R- Standard Amino Acids Phenylalanine(Phe), Tyrosine(Tyr), Glycine(Gly), Histidine(His) prepared in 10% aqueous solution of Isopropanol, 'U' urine sample, 'P' plasma sample and standard Amino Acids with Leucine(Leu), Serine(Ser), Valine(Val), Lysine (Lys), Threonine(Thr).

viz. L-Tyrosine and 1-dopa in phosphate buffer, and with Tyrosinase inhibitors Sodium diethyl dithiocarbamate and 4-Chlororesorcinol. Following incubation some hairs were washed in distilled water and mounted unfixed on slides in water and sealed. The Tyrosinase activity was estimated qualitatively under a dissecting microscope by evidence of darkening of cells in the lower matrix of anagen bulbs (16).

Alkaptonuria -The chief complaints of child with **Alkaptonuria** was scholastic backwardness and abnormal colour of urine which darkens on keeping for long time. Screening test showed black coloration with Benedict's test. 1-D paper chromatography of urine showed presence of Homogentisic acid with silver nitrate reagent. **Tyrosinuria** was found in one child at the age of 2 years. His plasma tyrosine levels were within normal limits. Screening test showed presence of tyrosine and its metabolites in urine by nitrosonaphthol test showing wine red color. 1-D paper chromatography of Urine showed tyrosine with Ninhydrin and Pauly's reagent. Similar findings were obtained with TLC. Neonatal Tyrosinemia is associated with increased excretion of tyrosine and its metabolites in 0.2 to 10% of neonates. It is more common in premature infants.

Xeroderma pigmentosum is an autosomal recessive condition with two major clinical forms one involving skin while

others show various neurological disorders. We have detected three cases with Xeroderma pigmentosum. All the three cases had dysmorphic features, short stature, Microcephaly and developmental delay with profound grade of retardation.

The most successfully intervened and well-managed condition is **congenital hypothyroidism**. Three children were found to have developmental delay with elevated TSH levels and low Thyroxin levels these levels were detected in other centers. Most of the children with this disorder were given thyroxin preparations and showed progress in their physical and mental development.

The aim of the study is to diagnose the cause of mental retardation which will help in the implementation of therapeutic measures in the affected child, and in case of the genetic cause being identified with high recurrence risk then prospective parents are suggested to go for prenatal diagnosis where by necessary preventive measures can be taken if fetus is affected. According to Peter Harper, Genetic counselling is the process by which patients or relatives at risk of a disorder that may be hereditary are advised of the consequences of the disorder, the probability of developing and transmitting it and of the ways in which this may be prevented or ameliorated (17). Genetic counselling plays very vital role in case of autosomal recessive conditions. Diagnosis of the condition makes it simpler to determine the risk factor associated with it. In autosomal recessive conditions, there is a significant increase in risk for the sibs of the affected child. We have detected a number of cases of autosomal recessive conditions where in two siblings are affected with the same disorder (Table III). Due to lack of awareness, many a times, parents do not approach or seek guidance from professionals. In spite of the first child being retarded, or sometimes misguided, they opt for a second pregnancy without the knowledge of the risk factor and land up with two children having same disorder.

So far more emphasis is put on the genetic and reproductive counselling and prenatal diagnosis whenever we talk of prevention of inherited diseases. Treatment of genetic disorder also should be equally important as it may extend from drug or dietary management, surgical measures to the future possibility of gene therapy. Hence knowledge of basic genetic defect, its expression, and its interaction with environmental factors greatly influences therapeutic interventions and future prospects of gene therapy for these disorders.

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