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Biochemical Screening Tests for the Detection of Inborn Errors of Metabolism in Children with Special Needs

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ABSTRACT

Inborn Errors of Metabolism [IEM] are caused by defect in the enzyme or in the transport of substances across the membrane. More than 3500 IEMs are believed to exist and nearly 700 IEMs have been identified till date. Significant association is reported between IEM and mental retardation. Screening is an important diagnostic tool for early diagnosis and initiation of treatment among newborn and mentally retarded children. A descriptive diagnostic study was carried out at Mangalore city to screen 150 children with mental retardation and developmental delay for inherited metabolic disorders using simple cost effective biochemical qualitative tests and thin layer chromatography for mucopolysaccharidosis, organic acidurias and inborn errors of amino acid, carbohydrate, heme and copper metabolism. The total occurrence was 27 [18%] cases of IEM including hyperglycinuria, hyperalaninemia, hyperornithinemia, histidinuria and galactosemia. Most common presentations were developmental delay, speech deficit and seizures. Significant association with proteinuria was observed. Most cases appeared to be sporadic. Male preponderance was observed. Initial detection of IEM among mentally challenged children was possible using simple, feasible, non invasive, biochemical screening tests. There is a need for national program to Screen newborns and MR children routinely using such simple tests as done in our study to reduce the burden of disease in our nation.

Keywords: Inborn errors, Inherited metabolic disorders, Mental retardation, Metabolic screening.

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INTRODUCTION

The term inborn errors of metabolism (IEM) was coined by Dr A.E. Garrod in 1908 for a group of genetically determined disorders that are caused by a enzyme deficiency or in the trans membrane transport of biological substances [1,3]. More than 3500 inherited metabolic disorders (IMD) are believed to exist and more than 700 are identified till date. Although rare, collectively they affect about 1-2 % of newborns and pose a significant health problem [2, 3]. Their incidence varies in different geographical regions and different ethnic groups. The occurrence of IEM is high in regions with greater incidence of consanguineous marriages [4]. Morbidity and mortality vary considerably. Some are relatively harmless (e.g. cystinuria and pentosuria), but some IMDs cause severe handicap (e.g. Phenylketonuria and Maple syrup urine disease) [2]. IEM can affect any organ at various stages of life, from newborn to adulthood depending on significant accumulation of toxic metabolites or on the deficiency. Most of the IEMs manifest with neurobehavioral manifestations and association of about 5.75% has been reported between mental retardation (MR) and IEMs [3-6].

Multiple studies have been conducted in India and abroad to screen neonates, MR children and pregnant women for IEMs. Most important IEMs detected by these studies include generalized aminoaciduria, PKU, mucopolysaccharidosis, methylmalonic aciduria, homocystinuria, biotinidase deficiency, alkaptonuria and maple syrup urine disease [7-16].

The overall prevalence of IEM in India is one in 2497 newborns and in Andhra Pradesh, one in 1000 newborns [17]. The prevalence of IEM is generally underestimated since a substantial proportion of cases remain under diagnosed. In India routine newborn screening is not carried out in spite of the availability of simple cost effective screening tests. Screening of newborn and mentally retarded (MR) children not only helps in early diagnosis and initiation of treatment, preventing permanent disability in the former and in improving quality of life in latter but also in providing prenatal diagnosis and counseling to parents. The primary role of screening thus appears to be preventive.

Our Present study aimed to screen mentally challenged children of Mangalore city for inborn errors of metabolism using a panel of simple biochemical tests and confirmed by thin layer chromatography.

MATERIAL AND METHODS

SOURCE OF DATA

A Descriptive diagnostic study was conducted on 150 special children with developmental delay and MR of age $\leq$ 16yrs from Mangalore city during February 2010 to May 2011. Children were selected using purposive sampling technique from special school and Father Muller Medical College Hospital. Patients with gastrointestinal illness and those with known acquired causes of mental retardation like birth trauma, infections etc were excluded. Informed Consent for performing tests on children was obtained from their parents. Study protocol was approved by the institutional ethical clearance committee. A Preformed questionnaire was given to parents in order to collect details including clinical

presentation, past history, developmental history, maternal history, birth history, family history, h/o consanguinity and drug history.

ASSAYS

Under aseptic precautions 25 ml random urine sample of these children was collected in sterile containers (preservative 2.5ml of 6N HCl was added in order to store the sample). The physical properties and preliminary chemical dipstick tests were performed using DX urine test-10 reagent strips from Piramal healthcare Limited [18-22]. Urine microscopy was performed on fresh sample using aqueous methylene blue and toluidine blue stains to observe for the presence of abnormal crystals and intracellular pathology (granules) [23]. Screening with biochemical tests including, Benedict's test for reducing substances, Ninhydrin test for amino acids, Ferric chloride test for phenolic compounds, Nitrosonaphthol test for tyrosine metabolites, Sulphuric acid test for indole derivatives, Isatin test for proline, Berry's Spot test (O-toluidine stain) and Cetylpyridinium chloride citrate turbidity test for Mucopolysaccharidosis, Dinitrophenylhydrazine test for keto acids, Methyl malonic acid test, Rothera's test for ketone bodies, Erhlich's aldehyde test for porphobilinogen, O-toluidine test for copper and Thin layer chromatography for aminoacids and carbohydrates were performed[23,24]. Special qualitative tests like mucic acid test for galactose was used to confirm the presence of the abnormal analyte in urine.

STATISTICAL ANALYSIS

The data was tabulated and analyzed by chi-square test and Fisher's exact test for nominal variables. Data was presented in the form of frequencies, percentages and diagrams (bar diagrams and pie charts).

RESULTS

Among 150 mentally challenged children 47% belonged to the age group of 11-15 years and only 6% were ≤5 years of age. Male children constituted 65% of the study population. Majority (84%) of the children were not evaluated for the cause of MR. Diagnosed cases were as in the table 1.

Table 1: Showing different diagnoses made in children with MR

Sl no	Diagnosis	Frequency
1	Undiagnosed	126
2	Down syndrome	12
3	Attention Deficit Hyperactivity Disorder	4
4	Autistic disorder	3
5	Cerebral palsy	3
6	Familial Hypercalciuria with cataract	1
7	Hydrocephaly	1
	Total	150

Twenty seven (18%) cases had some form of metabolic disorder. These cases had no statistically significant association with age or sex. Two cases of Anthocyaninuria, benign

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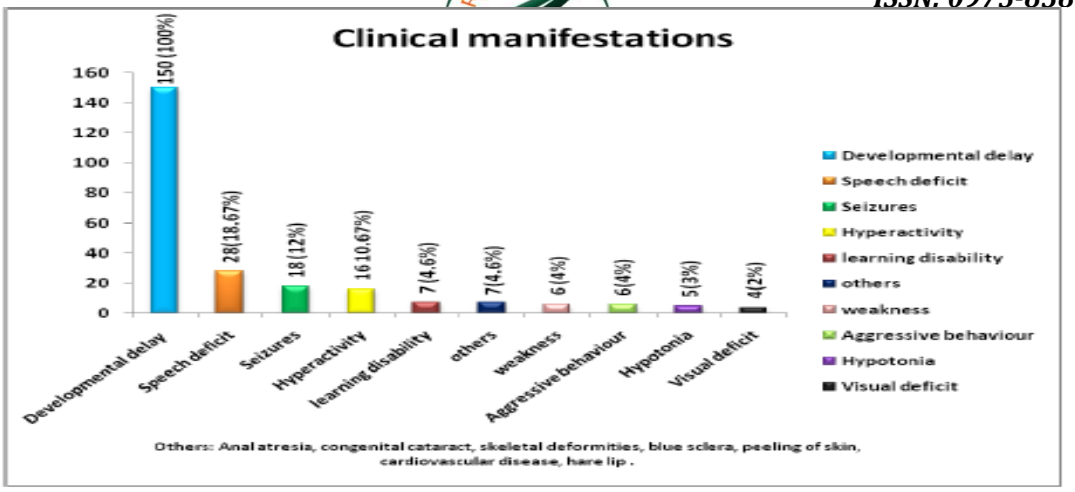

Fig 1: Column graph showing clinical manifestations in the study group

condition were also detected. The clinical presentation was highly variable - 53.3% of children presented since birth, 27.3% had onset at an early age (< 1yr) and 19.3% of the children had late onset of disease. The distribution of clinical features in these children is as depicted in the graph [Fig1]. Commonest associated clinical features other than developmental delay included Speech deficit and seizures. Severity of MR was significantly different [p<0.001] among the age groups. Majority had mild (48%) to moderate (36.7%) MR. The other parameters evaluated with respect to history of the child are shown in table 2. History of consanguinity was present in 12 % of the children with MR and 18.5% of children with metabolic disorder. It was not found to be statistically significant.

Table 2: Showing various clinical and sociodemographic parameters in mentally retarded children

Relevant Clinical History	Frequency positive out of 150	Percentage
Birth history with complications*	17	11.33%
Family history	4	2.66%
Consanguinity	18	12%
Maternal history[Antenatal/Postnatal] †	3	2%
Maternal medications during pregnancy	3	2%
Maternal Age > 30 yrs‡	32	21.3%
Paternal Age > 35 yrs§	43	28.7%
Drug history[Antiepileptics/Antipsychotics]	7	4.7%
Other illness in family	6	4%

* Birth complications: prolonged labor, prematurity, LBW, preeclampsia, cord round the neck etc.
† Maternal infection-1, Asthma-1, Fibroid uterus during pregnancy-1
‡ Maternal age > 35 yrs- 1 § Paternal age > 40yrs- 14

Biochemical tests performed on the urine of these special children and positive cases are shown in the table 3. Proteinuria was found in 13.3% of the children with MR. Eighteen children with IEMs had proteinuria which was statistically highly significant. Ketonuria and bilirubinuria also had significant association with aminoaciduria. Ninhydrin test (Fig 2) was strongly positive in 29 children of which 69% had proteinuria of various grades.

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Table 3: Showing various biochemical parameters performed for screening and statistical association with IEM tested using Chi square test and fisher's exact test.

Investigation	Frequency positive of 150 MR [Percentage]	Frequency among 27 IEM cases[percentage]
Dipstick tests		
Glucose	-	-
Protein	20[13.3%]	18[66.67%]*
Bilirubin	3	3†
Urobilinogen	-	-
Ketone bodies	3	3†
Blood/Hemoglobin	3	-
Leukocytes	2	2†
Nitrite	1	-
Test for glycosurias :		
Benedicts test	**7[4.7%]**	**6[22.2%]**†
TLC sugars	**2[1.3%]**	**2[7.4%]**†
Test for aminoacidurias and organic acidurias		
Ninhydrin test	29 [19.3%]	**27[100%]***
TLC Amino acids	**27[18%]**	**27[100%]**
Other tests for IEM	--	--

* P<0.001 [highly significant], † P<0.01 [significant]

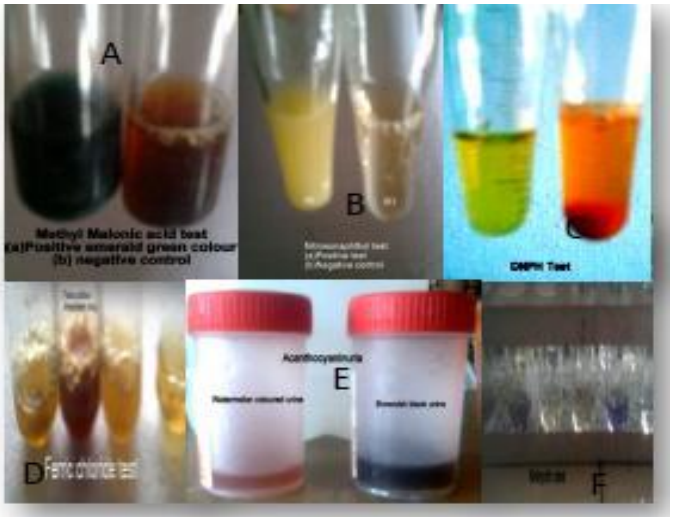

Fig 2 : Showing screening tests for IEM
A: Methymalonic acid test, B: Nitrosonaphthol test,C: DNPH test
D:Ferric chloride test, E: Anthocyaninuria, F: Ninhydrin test

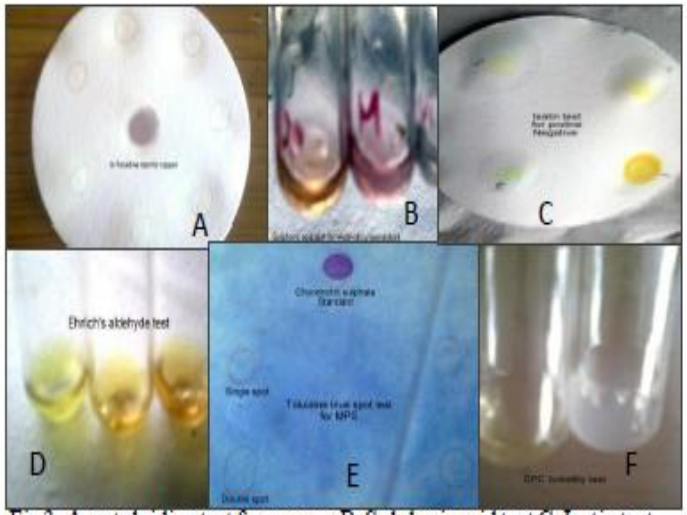

Fig 3: A: o-toluidine test for copper, B: Sulphuric acid test C: Isatin test, D: Ehrlich's aldehyde test, E:Berry's spot test, F: CPC turbidity test

TLC revealed aminoaciduria in twenty seven (18%) MR Children. Among them 19 cases of hyperglycinuria, 2 cases of histidinuria, 5 cases of hyperalaninemia, 1 case of hyperornithinemia were detected (Fig 4). No cases of Aromatic and branched chain aminoaciduria, ketoaciduria, Methyl malonic aciduria, Hydrindicuria, Porphyrias, Wilson's disease and Mucopolysaccharidosis were found in our study group (Fig 3). Benedict's test

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was positive in seven children with MR. TLC for reducing sugars was performed (Fig 4 D). Association with IEM was found in 6 children and was found statistically highly significant. Two children had Galactosuria. Other four cases probably had positivity due to non sugar reducing substance along with Glycinuria.

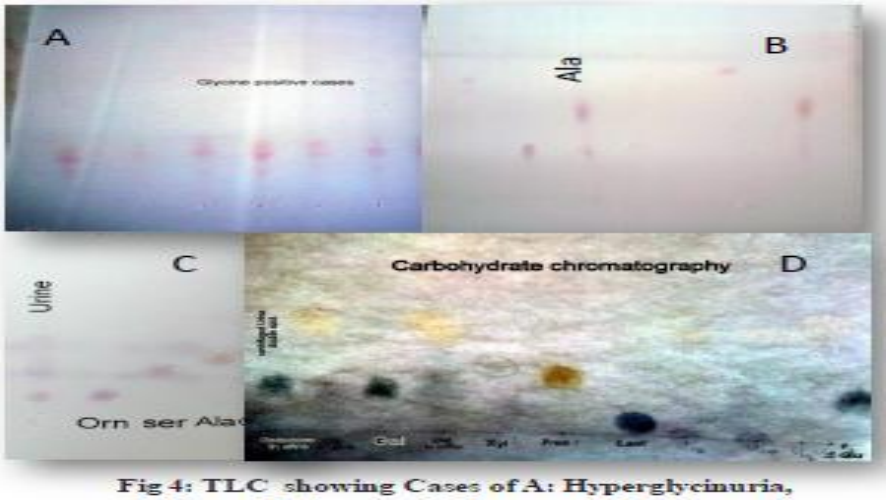

Fig 4: TLC showing Cases of A: Hyperglycinuria, B: Hyperalaninemia, C:Hyperornithinemia and D: Carbohydrate chromatography showing galactose in urine

DISCUSSION

Screening yields valuable information regarding the prevalence, the patterns of distribution and presentation of various types of IEM in that region. We screened 150 special children for inborn errors of amino acids, carbohydrates, metal (copper) and heme (porphyrias) metabolism, organic acidurias, storage disorders (mucopolysaccharidosis), transport defects (proteinuria, aminoacidurias etc) and also studied the various socio demographic parameters that can affect them. The total occurrence in our population was 18% (Table 4) and was high compared to previous studies. Male preponderance of cases was observed (61.5%) among IEM children and majority presented with developmental delay, speech deficits and seizures. Among the study group 126 (84%) children were undiagnosed. This can be attributed to non availability of facilities for diagnosis, poverty or due to ignorance.

Non parametric variables like age and sex of child, onset of disease, severity of MR, parental age, birth history, maternal history, family history and consanguinity had no association with total occurrence of the disease. Absence of family history indicates the sporadic occurrence of cases in this region. Consanguinity was observed in only 18.5% IEM cases and was not significant compared to other MR children.

Specific abnormal metabolites that are excessively excreted in urine of these children can be detected using a panel of simple, cost effective, qualitative, non invasive biochemical tests to diagnose IEMs. Preliminary investigations revealed a strong association of proteinuria, ketonuria and bilirubinuria with occurrence of IEM indicating that simple investigations also provide valuable additive information (renal and liver function and metabolic keto acidosis) useful for management of these children. Urine microscopy

although did not reveal any finding in our study, can be of diagnostic value if done bedside on fresh sample.

Aminoacidurias like hyperglycinuria, hyperalaninemia, histidinuria and hyperornithinemia were commonest IEM in our group. Association of all these aminoacidurias with mental retardation has been reported in earlier studies and case reports. Seventeen cases had isolated significant hyperglycinuria. However glycine excretion in traces is noted in healthy children as well. Considering this if we exclude the cases of hyperglycinuria the occurrence will be 6.6 % which is nearing the value obtained by Kumta et al (5.75%) [5] but slightly higher compared to other studies[15]. Hyperglycinemia is found to be one of the commonest IEM associated with MR according to some Indian studies. So we included cases which were strongly positive for glycinuria in our analysis. Cases of hyperglycinemia and hyperornithinemia are reported in earlier studies. Cases of Hyperalaninemia and histidinuria are hardly reported in India, but are reported in studies elsewhere. We also detected 2 cases of Galactosemia, an inborn error of Galactose metabolism by thin layer chromatography in our study group. However other IEMs including organic acidurias, hydrindicuria, porphyrias, mucopolysaccharidosis and also disorders of copper metabolism did not seem to be common in this region. There was high incidence of proteinuria (13.3%) among MR children which was found to be significantly associated with IEM (66.7%).

While screening, we also found three interesting cases, two cases of Anthocyaninuria which is a benign genetically determined disease where pigments (anthocyanins) present in fruits and vegetables is abnormally excreted in urine. One child's urine was pinkish red (watermelon) coloured and the other child's urine sample was dark brown in colour (Fig: 2E).They were confirmed as there was no metabolic abnormality and color disappeared in alkaline medium [22]. Another child had familial hypercalciuria with cataract which is an Autosomal dominant condition due to calcium sensory receptor gene (CaSR) mutation. Such cases mimic IEMs and therefore screening tests help in differential diagnosis.

Simple cost effective tests as done in our study thus provides an early diagnosis and facilitates early management if carried out routinely and on large scale in the form of new born screening.

CONCLUSION

Newborn screening program for IEMs is done routinely in many industrialized countries worldwide. In India, it is not mandatory even though there is a serious urge to have a national policy due to the ignorance and the apathy towards this aspect of health burden and also due to the fear of cost. Most cases remain undiagnosed as was evident in our group (84%).The possible reasons being poverty, lack of facilities, ignorance or due to misdiagnosis by clinicians as most IEMs mimic common ailments and present episodically. A high index of suspicion and routine screening for IEM among newborns as well as high risk children like mentally challenged may be useful for reducing the burden of disease in India.

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Table 4: Showing diagnosed cases of IEM in the study group by screening

Inherited metabolic diseases	Frequency	Percentage of 150[27]
Aminoacidurias		
Hyperglycinuria	17[+2] *	11.33[63]
Hyperalaninemia	5	3.3[18.5]
Histidinuria	2	1.3[7.4]
Hyperornithinemia	1	0.6[3.7]
Disorders of Carbohydrate Metabolism		
Galactosemia	2	1.3[7.4]
Total	27	18[100]

* 2 cases of Galactosemia also had Hyperglycinuria.

The distribution of various IEMs in different regions can be known only by routine screening. In our study we detected 27 cases with metabolic disorder among 150 children with MR /Developmental delay and found that disorders of intermediary metabolism especially of amino acids and carbohydrates are more common in our region. Such studies help in including only selective tests for screening so as to improve the sensitivity of the screening program which although nonspecific, largely excludes unaffected population. Confirmatory tests being costly can be limited to only those which are screening test positive.

Family history was insignificant in our cases suggesting sporadic occurrence of cases. The consanguinity in our study group was only 18.5%. Other studies have reported high incidence in South India and hence warrants routine screening in this region.

Screening helps in early diagnosis and initiation of treatment and rehabilitation and thereby prevents premature death, permanent neurological disability and facilitates follow up of future pregnancies for genetic counseling. There is a need to start a national program in India for routine screening of all newborns as in other countries worldwide, in order to decrease the morbidity and mortality due to IEM in future generation.

REFERENCES

[1] Garrod AE. The Croonian Lectures delivered before the Royal College of Physicians of London, in June, 1908. London: Oxford University Press; 1909.
[2] Pang CCP. Hong Kong Med J.1996; 2[3]: 264-273.
[3] Gupta A. To err is Genetics: Diagnosis and Management of Inborn Errors of Metabolism [IEM]: Chapter 32; 415-423. [homepage on the Internet]. [Cited 2009 Jul 28, 5:48PM]. Available from: http://www.krepublishers.com/06-Special%20Volume-Journal/T-Anth-00 Special% 20Volumes/T-Anth-SI-03-Anth-Today-Web/Anth-SI-03-32-Gupta-A/Anth-SI-03-32 Gupta-A-Tt.pdf.
[4] Uma SM, Jyothy A, Reddy PP et al. J Inher Metab Dis 1982; 5: 211-14.
[5] Datar CA, Apte BN. Bombay Hospital Journal 2008; 50[4]:560-65.
[6] Kumta NB, Indian J Pediatr 2005; 72:325-332.
[7] Choudhuri T, Sengupta S. Int J Hum Genet 2006; 6[1]:89-91.
[8] Rao SR, Narayan BS. Narayan et al. Indian J Med Res 1970; 58: 1753-57.
[9] Muhammad N, Muhammad M. Professional Med J 2007 ; 14(3):512-18.
[10] Narayanan HS, Rao SR, Rao MP et al. Indian J Psych 1988:30(2):123-27.

[11] Nagaraja D, Cristopher R, Subbukrishna DK ,et al. http://www.nimhans.kar.nic.in/ annreports/annreport0708.pdf .28/07/09,5:52 pm.
[12] KargerAG, Basel 1973. http://content.karger.com/ProdukteDB/produkte.asp?Aktion=ShowPDF&ArtikelNr= 152622&Ausgabe=237666&ProduktNr=224250&filename=152622.pdf .28/07/09:5:51 pm.
[13] Kaur M , Das GP and Verma CI . J Inher Metab Dis 1999 ; 17:230-33.
[14] Jailkhani R, Patil VS, Shivashankara AR, et al. J Clin Diagn Res 2008 ; 2 : 952-58.
[15] Bhatt C, Misra Z, Goyel N. Indian J Clin Biochem 2008; 23(1):10-16.
[16] Neonatal screening for inborn errors of metabolism using tandem mass spectrometry: experience of the pilot study in Andhra Pradesh, India. Indian J Pediatr. 2011 Aug; 78(8):953-960
[17] Latheef SA. Bioinformation 2010; 4(7):276-277.
[18] Fishbach FT, Dunning III MB. A Manual of Laboratory and Diagnostic Tests. 8th ed. Philadelphia: Lippincott Williams & Wilkins; 2008.
[19] Patel HP. Heidelberg: Springer; 2008.
[20] Henry JB, Clinical Diagnosis and Management by Laboratory Methods. 20th ed. Philadelphia, PA: W.B. Saunders Company, 2001.
[21] Varley H, Gowenlock AH & Bell M Practical Clinical Biochemistry: Vol-I.5th ed. London: William Heinemann Medical Books Ltd; 1984.
[22] Neil RM, Buist. Brit med J1968; 2:745-49.
[23] ICMR Task Force On Inborn Metabolic Disorders: Annexure X: Biochemical Screening Tests. [Homepage on the Internet]. 2007 [cited 2011 Aug 13]. Available from: http://icmrmetbionetindia.org/pdf/annexure%2010.pdf