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Original Article

Biochemistry Section

Prevalence of Inborn Errors of Metabolism in Neonates

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ABSTRACT

Introduction: Among the most advanced public health promotion and disease prevention programs, the newborn screening is of paramount importance, seeking timely detection, diagnosis and treatment of genetic disorders which may otherwise lead to serious consequences upon the health of newborn.

Aim: To evaluate the prevalence of Inborn Error of Metabolism (IEM) disorders among neonates of various ethnic or racial groups from east, west, north and south, zones of India through newborn screening.

Materials and Methods: A cross-sectional, population based prospective study was conducted at PreventiNe Life Care Laboratories, Navi Mumbai, Maharashtra, India. Study was conducted for a period of three years from October 2012 to November 2015. Mass screening of newborn blood samples was done via TMS/GCMS/Enzyme assay/HPLC/ELISA technique. The blood and urine samples were used for analysis. The samples have been collected from 150 locations through various hospitals across India. Samples obtained were categorised zone wise (east, west, north, south zones of India). For analysis of blood, samples were collected by heel prick method.

Results: In the present study, 2.9% prevalence (of the total 70,590 samples analysed, 2053 cases were found positive) of IEM was observed. Of these positive cases, 13% (279 of 2053 positive cases) cases belonged to eastern zone, 24% (493 of 2053 positive cases) were from northern zone, 38% (793 of 2053 positive cases) were from southern zone and 23% (488 of 2053 positive cases) were from western zone. Among these, the highest prevalent disorder was found to be G6PD deficiency, with 1.3% (923 positive of 70,590) cases reported followed by haemoglobinopathies, 0.5% (360 positive of 70,590) and congenital hyperplasia with 0.34% (239 positive of 70,590) cases of the total newborns, screened.

Conclusion: The newborn screening is expanding its wings throughout the world. The outcome of present data offers a unique opportunity to explore the birth prevalence of inborn metabolic disorders in the current population. Understanding the birth prevalence of these disorders in India from its various zones will definitely improve the short term and long term medical needs faced by affected communities.

Keywords: Heel prick method, Metabolic errors, Neonatal disorders, Newborn screening

INTRODUCTION

Inborn errors of metabolism disorders are a complex and heterogeneous group of disorders due to enzymatic defect in single pathway of intermediary metabolism. Certain pathological alterations in normal catabolic path of amino acids, carbohydrates, lipids or biogenic amine often cause abnormal excretion pattern of organic metabolites. These metabolites are normally absent or present in very small concentration [1,2]. The inborn metabolic disorders currently in human beings exceed more than 500 types and of these 100 alone are the disorders of the amino acid metabolism [3]. Being an important cause of morbidity and mortality in clinical practice and any delay in the diagnosis and treatment of these disorders leads to a variety of symptoms including moderate to severe neuropsychological manifestations in the form of mental retardation, seizures, death etc., [4]. Neonatal disorders presently are the major cause of perinatal and neonatal mortality with 9.2% cases alone in urban areas of India [5]. The newborn screening is a technique in our hands to investigate congenital genetic and metabolic disorders in order to prevent the mortality and disabilities associated with these disorders. It is a study meant to screen infants shortly after birth for a list of conditions that are treatable. Symptoms of these disorders are not clinically evident in the newborn period. Some of the conditions included in newborn screening programs are only detectable after irreversible damage has been done. But in many of the cases sudden death is the first manifestation of the disease [6]. Therefore, in present study, mass screening of the blood samples of newborns was done via TMS/GCMS/HPLC/Enzyme assay technique [7,8]. The blood samples for analysis were collected by heel prick method. Both blood and urine (freshly voided urine) samples were collected on filter paper.

MATERIALS AND METHODS

A cross-sectional, population based prospective study was conducted at PreventiNe Life Care Laboratories. Study was conducted for a period of three years from October 2012 to November 2015. A total of 70,590 neonatal blood and urine samples were analysed for IEM. The samples have been collected from 150 locations through various hospitals across India. These neonates were already enrolled for the cord blood and cord tissue banking and newborn screening was an additional test offered to parents as routine screening to rule out IEM. The neonates belonged to various racial and ethnic groups of India. Samples obtained were categorised zone wise (east, west, north, south zones of India) and processed at Central Laboratory at Navi Mumbai, India. The blood samples for analysis were collected by heel prick method [7]. A pinprick puncture in one heel of the newborn was done and blood was soaked into pre-printed collection cards or Guthrie cards. Urine samples were also collected on filter paper by soaking freshly voided urine. Blood and urine samples were collected in all the cases. Tests done on blood samples were for beta thalassaemia, sickle cell anaemia (HbSS), sickle cell disease (HbS/C), variant haemoglobinopathies (C,D,H barts band), including HbE, congenital hypothyroidism, congenital hyperplasia.

Rest of the tests were performed using urine samples. Samples were analysed for 119 disorders including amino acid disorders, fatty acid disorders, organic acid disorders, carbohydrate disorders, peroxisomal disorders and various other disorders using Tandem Mass Spectrometry (TMS), Gas Chromatography-Mass Spectrometry (GC-MS), High Performance Liquid Chromatography (HPLC), Enzyme Assay and Enzyme linked Immunosorbant Assay (ELISA) method [Table/Fig-1,2] [7,8]. Written informed consent from parents was taken before collection and analysis of the samples. Ethical clearance was obtained from ethical clearance committee of Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh, India. For sample collection, baby was prepared to have 3-4 milk feeds. As stress of birth may change various values especially the thyroid profile, collection of samples for IEM screening during initial 24 hours was avoided. Infants on antibiotics, premature babies or babies on blood transfusion were excluded from the study.

Test Done on Blood Samples	
Haemoglobinopathies	
S. No. 1	BetaThalassaemia
2	Sickle Cell Anaemia (HB SS)
3	Sickle Cell Disease (Hb S/ C)
4	Variant Haemoglobinopathies(C, D, H, bart band), including HbE
Endocrinology	
5	Congenital Hypothyroidism
6	Congenital adrenal Hyperplasia
Other Genetic Disorders	
7	Cystic Fibrosis
8	G6PD Deficiency
Test Done on Urine Samples	
Amino acid disorders	
9	Phenyl ketonuria
10	Defect in Biopterin Cofactor Biosynthesis
11	Defects in Biopterin Cofactor Regeneration
12	GTP Cyclohydrolase (GTPCH) Deficiency
13	Dihydropteridine Reductase Deficiency
14	Benign Hyperphenylalaninaemia (H-PHE)
15	Tyrosinaemia Type I
16	Tyrosinaemia Type II
17	Tyrosinaemia Type III
18	Trasient Tyrosinaemia in Infancy
19	Tyrosinaemia caused by liver dysfunctions
20	Maple Syrup Urine Disease (MSUD)
21	Carbamoyl Phosphate Synthetase-1 Deficiency
22	Ornithine Transcarbomylase (OTC) Deficiency
23	Citrullinaemia
24	Citrullinaemia Type II
25	Argininosuccinic aciduria
26	Argininaemia
27	Hypermethioninaemia
28	Homocysteinuria
29	Alkaptonuria
30	Tryptophanuria with dwarform
31	Xanthurenic Aciduria
32	Valinaemia
33	Hyperleucinaemia
34	Dihydroptoyl Dehydrogenase deficiency
35	3-Hydroxylbutyryl CoA Deacylase Deficiency
36	Histidinuria
37	Hurtnup Disease
38	Lysinuric Protein Intolerance
39	Famillial Renal Iminoglycinuria
40	Iminoglycinuria
41	2-Ketoadipic aciduria
42	Sacchropenuria
43	Hydroxylysinuria
44	Cystathionuria
45	Hyperprolinaemia
46	Hyperprolinaemia Type II
47	Hyperhydroxyprolinaemia
48	5-Oxoprolinuria
50	Hypersarcosinaemia
51	Imidazole aminoaciduria
52	Formiminoglutamic aciduria
53	Serum carnosinase deficiency
54	Glutathionuria
55	Hyperpipecolatemia
56	3-Aminobutyric aciduria
57	Histidinaemia
Organic acid Disorders	
58	Propionic acidemia
59	Multiple carboxylase Deficiency
60	Methyl Malonic Acidemia
61	Methyl Malonyl CoA Mutase Deficiency
62	Methyl Malonic Aciduria
63	Malonic Acidemia
64	Biobutyryl CoA Dehydrogenase Deficiency
65	MethylButyryl CoA Dehydrogenase Deficiency
66	Methyl Malonic Saemialdehyde Dehydrogenase Deficiency
67	B-Ketothiolase Deficiency
68	Isovaleric aciedmia
69	3-MethylcrotonylCoA Carboxylase Deficiency
70	3-Methyl Glutaconic aciduria
71	3-Hydroxy 3-methyl Glutaric Aciduria
72	Glutaric aciduria Type-II
73	Glutaric aciduria Type-I
74	Mevalonic Acidemia
75	3-Methyl 3-Hydroxy Butyric Aciduria
76	4-Hydroxybutyric aciduria
Carbohydrate Disorders	
77	Galactosaemia
78	Galactokinase Deficiency
79	Galactose Epimerase Deficiency
80	Transient Galactosaemia
81	Fructosuria
82	D-Glyceric Aciduria
83	Fructose 1, 6 Diphosphatase Deficiency
84	Endogenous Sucrosuria
85	Lactose Intolerance
Fatty Acid Oxidation disorders	
86	Short Chain CoA Dehydrogenase deficiency(SCAD)
87	Medium Chain CoA Dehydrogenase Deficiency(MCAD)
88	Long Chain CoA Dehydrogenase Deficiency (LCAD)
89	Very Long Chain CoA Dehydrogenase Deficiency (VLCAD)
90	Short/Medium Chain 3-Hydroxy CoA Dehydrogenase Deficiency

91	Long Chain 3-Hydroxy CoA Dehydrogenase Deficiency
92	Mitochondrial Trifunctional Protein Deficiency
93	Carnitine Transport Defect
94	Multiple CoA Dehydrogensae Deficiency
95	Medium Chain Ketoacyl CoA Dehydrogenase Deficiency
Peroxisomal Disorders	
96	Zellweger Syndrome
97	Neonal Adenoleucodystrophy
98	Infantile Refsums Disease
99	Zellweger Like Syndrome
100	Primary Hyperoxaluria
Disorders of Purine Pyrimidine Metabolism	
101	Adinosine Deaminase Deficiency
102	Lesch Nyhan Syndrome
103	Partial Deficiency ofHypoxanthine Adenine Phosphoribosyl Transferase
104	Adenine Phosphoribosyl Transferase Deficiency
105	Xanthinuria
106	Orotic Aciduria
107	Thymine uraciluria
108	Dihydropyriminidase Deficiency
109	Hyperuric Acidemia
Lactic Acidemia, Hyperpyruvic Acidemia	
110	Pyruvate Dehydrogenase Deficiency
111	Pyruvate Dehydrogenase Phosphatase Deficiency
112	Pyruvate carboxylase deficiency
113	Pyruvate decarboxylase deficiency
114	Leigh Syndrome
Other IEM	
115	Biotinidase Deficiency
116	Canavan Deficiency
117	Fumerate Hydrolase Deficiency
118	Hyperornithinaemia-Hyperammonaemia-Hyperhomocitrullinaemia (HHH) Syndrome
Miscellaneous genetic condition	
119	Neuroblastoma

[Table/Fig-1]: Test done on blood and urine samples.

Dried Blood Sample (DBS) Analysis on Tandem Mass Spectrometry

The DBS samples were analysed for amino acid and acyl carnitine profile on the system LCMS-MS 8030, Triple-Quadruple Mass Spectrometer equipped with ESI probe (Shimadzu, Japan). DBS samples were processed according to the method stated in Chromsystems reagent kit [9]. With the every batch of patients being tested, a positive and negative control sample was made and analysed. The data analysis was performed on Neonatal Software version 3.2, and the markers were expressed as µmol/L based on its ratio with stable-isotope deuterium-labeled isomer (e.g., Leucine/deuterium-Leucine). Reference values for amino acid and acyl carnitine profiles have been calculated from the DBS concentrations measured in healthy newborns and early infancy.

Urine Analysis by GCMS

For urine filter paper sample analysis, T. Kuhara's method was followed [10]. Samples were extracted with water and pretreated with urease at 37°C to remove urea followed by deproteinisation with ethanol containing Heptadecanoic acid as internal standard. Samples were then vacuum dried and residues were derivatised by adding N, O,-bistrimethylsilyl) trifluroacetamide (BSTFA) and Trimethylchlorosilane (TMCS). Further, 1 µL Aliquots of derivatised samples were injected into Shimadzu QP-2010 Plus GC/MS using auto sampler in split mode. Analysis of metabolites was conducted chromatographically, using trimethylsilyl derivatised compounds. The data analysed with computer-assisted program and National Institute of Standards and Technology (NIST) library.

Enzyme Linked Immunosorbant Assay

An assay based on sandwich Enzyme Linked Immunoassay (EIA), using peroxidise labelled anti TSH monoclonal antibody in a microwell with coating of another anti TSH monoclonal antibody, was used for diagnosis of congenital hypothyroidism. For microplate neonatal TSH estimation, 1/8 disc was punched out from the blood collection card. Intensity of colour in the microwell was proportional to TSH concentration [11].

High Performance Liquid Chromatography

For Amino acid analysis, Thin Layer Chromatography is used as a preliminary screening technique using Butanol, Acetic acid, Water and staining with ninhydrin. Confirmation of screen positive cases was carried out by reverse-phase HPLC following the pre-column derivatisation with Phenyl isothiocyanate (PITC) [11,12].

STATISTICAL ANALYSIS

All the obtained data were calculated and represented by using Microsoft excel 2007.

RESULTS

With 2.9% prevalence of IEM, out of total 70,590 cases of neonates screened, 2053 cases (including both male and female neonates) were found positive. Neonates were screened for total 119 disorders [Table/Fig-1]. The most prevalent disorder was found to be G6PD deficiency with 923 (1.3% of total screened 70,590 cases) cases positive with 44% prevalence (923 of 2053 positive cases) followed by 360 (0.5% of total screened 70,590 cases) cases with 17.5% prevalence (360 of 2053 positive cases) of haemoglobinopathies and the number of congenital hyperplasia and congenital adrenal hyperplasia were 239 (0.34% of total screened 70,590 cases) and 118 (0.16% of total screened 70,590 cases) of the total cases screened for NBS with prevalence of 11.6% (239 of 2053 total positive) and 5.7% (118 of 2053 positive) respectively. Various amino acid disorders screened were citrullinaemia-I, homocysteinuria, hypermethioninaemia, Maple Syrup Urine Disease (MSUD), tyrosinaemia Type I, II, III, phenylalaninaemia etc., and are clearly mentioned in [Table/Fig-1]. Number of all positive cases are shown in [Table/Fig-2]. Highest number of cases was of MSUD (26) with 1.2% prevalence (26 out of 2053) followed by phenylketonuria and alkaptonuria with 0.3% and 0.2 % prevalence (7 and 6 of 2053 positive cases). Among fatty acid disorders, highest number of cases (7) of Carnitine Uptake Defects (CUD) was found with 0.3% prevalence (7 out of 2053 positive). One case each of Medium Chain Acyl-Coa Dehydrogenase Deficiency (MCAD), carnitine palmitoyl transferase deficiency Type-I and 2 cases of Short Chain Hydroxyl-Acylcoa Dehydrogenase Deficiency (SCHAD) were observed with 0.04% (1 of 2053 positive) and 0.09 % (2 out of 2053 positive) prevalence respectively [Table/Fig-2]. Rest of the disorders and number of positive cases are mentioned in [Table/Fig-1,2]. Other disorders with significant number of cases were glucose 6 phosphate dehydrogenase deficiency with 44% prevalence (923 of 2053 positive), cystic fibrosis with 3.4% prevalence (71 of 2053 positive cases) and biotinidase deficiency with 1.9% prevalence (41 of 2053 positive) and others disorders are shown in [Table/Fig-2]. Zone wise distribution of the cases are clearly mentioned in [Table/Fig-1,2]. Also, clear account of use of techniques done, for detection of the diseases are presented in [Table/Fig-1,2].

Technologies	East	North	South	West	Number of cases
Enzyme linked immunosorbant assay	**n=45**	**n=90**	**n=233**	**n=61**	**n=429**
Congenital adrenal hyperplasia	17	35	53	13	118
Congenital hypothyroidism	21	50	130	37	238
Congenital hypothyroidism and beta-thalassaemia			1		1
Cystic fibrosis	7	4	49	11	71
Liver disease or hyperalimentation and/or ketoacidosis		1			1
Enzyme assay	**n=165**	**n=212**	**n=340**	**n=254**	**n=971**
Biotinidase deficiency	12	9	14	6	41
G6PD deficiency	153	201	321	247	922
Galactosaemia		2	3		5
Hyperphenylalaninaemia			1		1
Phenylketonuria			1	1	2
Gas chromatography mass spectrometry	**n=6**	**n=75**	**n=53**	**n=49**	**n=183**
2-Hydroxyglutaric aciduria			2		2
3-Methylcrotonyl CoA carboxylase deficiency			1		1
3-Methylglutaconic aciduria			1		1
4-Hydroxybutyric aciduria		1			1
Alkaptonuria	1	2	1	2	6
Beta-keto thiolase deficiency		2		2	4
Biotinidase deficiency or multiple carboxylase deficiency			1		1
Canavan disease		1			1
Ethyl malonic aciduria		1			1
Galactosaemia	1	1	1	1	4
Glutaric aciduria			1		1
Glutaric aciduria Type I		13	5	6	24
Glutaric aciduria Type II	1	1	1	1	4
Hyperglycinuria (nonketotic)		2			2
Hyperornithinaemia (with Gyrate Atrophy)			1		1
Hypersarcosinaemia		1			1
Isovaleric acidemia			2	1	3
Lactic aciduria			1		1
Lactic aciduria (primary)			1		1
Lactose intoelrance			1		1
Maple syrup urine disease	1	2	3	5	11
Medium chain acyl CoA dehedrogenase deficiency			1		1
Medium chain acyl CoA dehedrogenase deficiency or 2-Hydroxyglutaric aciduria			1		1
Methyl malonic acidemia	2	22	12	13	49
Mitochondrial disorder		6	4	7	17
Mitochondrial disorder (or) primary lactic acidemia				1	1
Multiple acyl dehydrogenase deficiency		1			1
Multiple carboxylase deficiency		1			1
N-acetylglutamate synthase deficiency			1		1
Orotic aciduria				1	1
Phenylketonuria		2		2	4
Propionic acidemia		7	7	4	18
Pyroglutamic aciduria		1			1
Pyruvate dehydrogenase (E1) deficiency		1			1
Succinate saemialdehyde dehydrogenase deficiency		2	1		3
Thymine uraciluria			2		2
Tyrosinaemia		2		1	3
Urea cycle disorder		3	1	2	6
High performance Liquid chromatography	**n=58**	**n=62**	**n=139**	**n=101**	**n=360**
Haemoglobinopathies	58	62	139	101	360
Tandem mass spectromretry	**n=5**	**n=54**	**n=28**	**n=23**	**n=110**
3-Hydroxy-3-Methylglutaryl-CoA lyase deficiency HMG			1		1
3-Methylcrotonyl CoA carboxylase deficiency		1			1

3-Methylcrotonyl CoA carboxylase deficiency or mitochondrial acetoacetyl-CoA thiolase deficiency			1		1
Argininaemia		2			2
Argininosuccinic acidemia		1			1
Benign hyperphenylalaninaemia		1			1
Benign hyperphenylalninaemia (or) biopterin cofactor deficiency		1			1
Beta-keto thiolase deficiency		2	2		4
Biotinidase deficiency or multiple carboxylase deficiency			1		1
Biotinidase deficiency or multiple carboxylase deficiency or 3-Methylcrotonyl CoA carboxylase deficiency		1			1
Carnitine acylcarnitine translocase deficiency			1		1
Carnitine uptake defect		2	1		3
Carnitine uptake defect or carnitine palmitoyl transferase deficiency Type II (CPT-II)		1			1
Carnitine uptake defect or primary carnitine deficiency		1		2	3
Citrullinaemia		3			3
Citrullinaemia or arginosuccinic acidemia				1	1
Citrullinaemia Type I		1			1
Citrullinaemia Type I or citrullinaemia Type II or argininosuccinic academia		1			1
Classical hyperphenylalaninaemia or biopterin cofactor deficiency		1	2		3
Fatty acid oxidation disorder			1		1
Glutaric aciduria Type I		7	3	2	12
Glutaric aciduria Type I or mitochondrial disorder				1	1
Glutaric aciduria Type II			3		3
Homocystinuria and/or hypermethioninaemia		1			1
Hyperalimentation				1	1
Hyperalimentation (or) liver disease		2			2
Hypermethioninaemia or liver disease	1				1
Isobutyryl-CoA dehydrogenase deficiency or short-chain Acyl-CoA dehydrogenase deficiency		1			1
Isovaleric acidemia			2	1	3
Isovaleric acidemia or 2-Methylbutyryl-CoA dehydrogenase deficiency		1			1
Ketoacidosis			1		1
Maple syrup urine disease		11	1	3	15
Maple syrup urine disease or propionic acidemia		1			1
Medium chain acyl CoA dehedrogenase deficiency or medium chain triglyceride Oil	1				1
Methyl malonic acidemia (MMA) (or) propionic aciduria	2	4	4		10
Methylmalonyl-CoA mutase deficiency (or) propionic acidemia			2		2
Mitochondrial disorder				1	1
Mitochondrial disorder (or) hyperalaninaemia		1			1
Neonatal carnitine palmitoyl transferase deficiency Type I		1			1
Phenylketonuria (or) biopterin cofactor deficiency		1			1
Phenylketonuria (PKU)		1			1
Primary carnitine deficiency			1	1	2
Propionic acidemia (PA)		2	1	5	8
Short chain hydroxy acyl-CoA dehydrogenase deficiency				2	2
Tyrosinaemia I/II/III/transient				1	1
Tyrosinaemia Type I	1				1
Urea cycle disorder				2	2
Very long chain acyl-CoA dehydrogenase deficiency		1			1
Grand total	279	493	793	488	2053

[Table/Fig-2]: Test done on blood and urine samples.

DISCUSSION

There was significant number of positive cases seen in series of neonatal tests. Similar data in the local population is practically rare for comparison though very few such studies have been conducted and reported in other states. In current scenario there is basic and stern requirement for a screening program, to avail the epidemiological data regarding disease burden. In India, the birth rate is 21.76 births/1,000 population [13]. Delhi alone has nearly 900 births, reported every day and out of these one or two babies are born with a metabolic defect [14]. However, the diagnosis is ignored due to lack of awareness and easily available techniques. Also, because present health policies are more concerned towards prevention of mortality and that figure has of course rectified but there has been a simultaneous hike in number of cases of disabilities too. In this newborn screening study carried out among 70,590 neonates, G6PD disorder was found to be most prevalent with 1.3% (923 cases of 70590 screened) of all the cases screened and with 44% prevalence (923 of total 2053 positive cases). The

next most common disorder was haemoglobinopathies with 0.5% (360 cases) and with 17.5% prevalence (360 cases of 2053 positive cases) followed by 0.34% (239 cases of 70590 screened) cases of Congenital Adrenal Hyperplasia (CAH) with 11.6% (239 of 2053 positive cases) prevalence and 0.16% (118 cases of 70590 screened) of Congenital Hypothyroidism (CH) with 5.7% (118 cases of 2053 positive cases) prevalence. In one of the major study conducted among 125 thousand neonates, have reported homocysteinaemia, hyperglycinaemia, MSUD, phenylketonuria (PKU), congenital hypothyroidism and G6PD deficiency to be the most common disorders with good prevalence [15]. A study done in year 2014 have documented CH, CAH, G6PD, Biotinidase deficiency, galactosaemia and cystic fibrosis as the most prevalent disorders [16]. In a hospital population based study, 2479 neonates were screened for G6PD deficiency, and had a 28.3% incidence in males and 1.05% in female neonates [17]. Considered as most life threatening disorders, haemoglobinopathies are a group of a number of disorders including β-thalassaemia, sickle cell anaemia etc. According to WHO nearly 10,000 babies in India are born every year affected with β-thalassaemia [18]. Tribal community which represents our 8% Indian population, predominantly suffer from sickle cell anaemia [19]. Significant number of cases of haemoglobinopathies was reported in present study. In one more study conducted at AIIMS, CAH was diagnosed in about 38% children presenting with ambiguous genitalia [20]. So outcome of present study are compatible with earlier reports documented. Infact for CH, CAH and glucose-6-phosphate dehydrogenase deficiency, neonatal screening has been proposed to be must in Indian scenario. In one of the study conducted by Gopalkrishnan V et al., done in Lucknow, Uttar Pradesh in 2014, cut-offs for galactosaemia and biotinidase deficiency were 0.32% and 0.16%, respectively while in present study the cut-off values were found to be 0.43% and 1.9% [16]. Cystic fibrosis cases have been reported rare in Indian population with prevalence 1/43,321 to 1/100,323 [19]. In a study conducted at Hyderabad also, have shown maximum prevalence of cystic fibrosis, galactosaemia and biotinidase deficiency beside other disorders including cases of urea cycle disorders [15]. Mitrochondrial disorders were less than 1%. From the analysis of various studies it is apparent that a significant portion of the population is being affected from metabolic disorders. While before it can be recommended to be included in a nationwide screening program, lot many studies are needed to be done. In present study an attempt is also made to understand the geographical distribution of the IEM among newborn, zonal categorisation of the positive cases has been done. Zonal frequency was found with maximum cases found in southern zone with 38.5% (793 cases of 2053 positive) prevalence, northern zone 24.2% (493 cases of 2053 positive) prevalence, west zone 23.7% (488 cases of 2053 positive), and in eastern zone 13.57% (279 cases of 2053 positive) prevalence of IEM in India. Such type of zone wise presentation of NBS data is rarely reported in the literature in India. In the last few years India has immensely progressed in the field of medical science and technology. Laboratory advancement especially in mass spectrometry has facilitated so much easier screening of newborns for many IEM. This is the need of time for all of us to be aware of the fact that early detection, appropriate investigation and treatment can prevent the morbidity and mortality in neonates. A number of such studies are also needed to be done to determine actual prevalence of disorders in different parts of India. Though, in Asia Pacific region, there are clear evidences of growth of newborn screening, well reported in the literature. Newborn screening through blood spot began in New Zealand and Australia in the year 1960, followed by Japan and then in Singapore through cord blood screening for G6PD. Screening for congenital hypothyroidism as an additional screening started in 1980 followed by development of new programmes started in countries like Taiwan, Hong Kong, China, India, and Malaysia [22]. During 1990's various new programmes developed based on previous experience especially in Korea, Thailand and Philippines with rapid growth. In the year 2000's countries including Indonesia, Magnolia, Sri Lanka, Myanmar and Pakistan with limited funding from the International Atomic Energy Agency, started a screening programme for congenital hypothyroidism [21]. Recently Palau and Philippine jointly started NBS programme while there is very less information available on newborn screening activities in Nepal, Cambodia, Laos and the other Pacific Island nations [21,22]. According to the current data, around 131 million babies are born every year across the globe and approximately 7.9 million are born with IEM [22]. Nearly half of the births and defects occur in Asia Pacific region and almost 80% of these occur in China, Indonesia, Bangladesh, India, and Pakistan [23]. Japan has expanded the NBS program through funding support for IEM of aminoacids, organic acids, fatty acid metabolism in the year 2012 while in other Asian countries patients generally pay for these cost effective testing [24]. Taiwan, Japan and Korea have recently reported new born screening of Lysosomal disorders and Pompe disease [25-27]. From the limited data available of NBS in the Asian countries, an apparent high incidence of CAH has also been reported [28]. In present study also the 3.1% prevalence has been observed. As far as NBS initiation and implementation program in developing countries, especially South-East Asian is concerned, it is running at very slow pace and a very challenging job here. Infact in most of the countries, NBS is not mandatory and has not yet been incorporated into the public healthcare system [28,29]. Limited funding, manpower shortages, inadequate support services, low public awareness are the main challenges which nations have to face. Though Laws requiring new born screening or its offering now well established in some of the countries and significant efforts are being made to include NBS as national health insurance maternity benefits packages. Asia Pacific region is reported have half of the births in the world. So in order to maintain the health status of the children, proper implementation and expansion of NBS is very much required [30].

LIMITATION

We were unable to produce separate male and female data of the diseases. Also, we have not discussed the conditions where two or more diseases may possibly be present in the same patient.

CONCLUSION

NBS has been established as important tool to detect inborn errors of metabolism. In order to curb the incidence of congenital anomalies, World Health Organisation has recommended for new born screening. However, current state of NBS is not very good in Asian countries. The main challenges in establishing this program have been the lack of funds, less awareness in public, inadequate manpower and support services. Early diagnosis may save a number of children from getting disabled. In fact presymptomatic evaluation of these conditions through NBS will definitely minimise the irreversible, life threatening changes and will thereby improve the treatment regimen. A significant number of positive cases were found in present screening of new born babies, which apparently shows that IEM disorders are quite prevalent in Indian population. The present study put forward many facts about demographic prevalence in India but many such studies are required to be done in the direction so as to establish the actual burden of IEM, their various types and strategies to cop up with these situations.

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