Incidence and spectrum of Inborn Errors of Metabolism in Critically Ill New Borns in India

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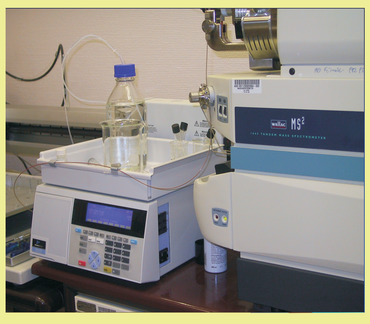
Introduction :- India is a developing country with population of over 1.2 billion with annual birth rate of over 25 million. We do not have any nation wide screening program as yet. We also have high rate of consanguineous marriages, which increases the risk of autosomal recessively inherited disorders including some of the inborn errors of metabolism (IEMs). Incidence of IEM as per international data suggests approx 1: 1000 births, and therefore we can expect atleast 25,000 babies born with screenable IEMs.

Objectives : - Our objective was to determine the spectrum of IEMs in critically ill babies so as to recommend screening tests to be performed atleast for NICU babies

Subjects and Method :- We investigated 901 critically ill babies admitted from various NICUs with a clinical suspicion of IEM for a period of Jan 2000 - Sep 2011. All these babies were subjected to a standard panel of tests to detect IEM – Plasma ammonia, Lactate, Blood sugar levels, ABG, Anion Gap, Urine Ketones, MRST, urine Orotic Acid, HPLC Aminoacids of plasma and if required CSF, Urine, GC - MS for Organic acids (Fig. 1) and MS / MS for Carnitine / Acylcarnitine (Fig. 2) and Aminoacid Profile. In addition to these some other specific diagnostics tests were performed in relevant cases e.g. 17 OHP, VLCFA analysis ,Transferrin Iso - electric focusing to rule out CDG, mutation analysis for Cystic Fibrosis, MTHFR etc.

Fig. 1 :- GC-MS machine

Fig. 2 :- Tandem Mass Spectrometry Machine



Results : - We investigated total 901 cases of CINB, out of which we could complete the investigations of 726 cases. We are presenting 25 most common disorders whose incidence in this cohort is as follows :

 # Table 1: Study period Jan 2000 – Sept. 2011

Total Cases Of CINB	901
Incompletely Investigated	175
Final Diagnosis Arrived	726

Discussion : - Though individually IEMs are considered rare, collectively they are common. A neonatologist is bound to see critically ill new borns with suspected IEMs rather frequently if the index of suspicion is high. In our cohort we found 5 most common conditions to be NKHG (5.1%), Galactosemia (3.9%), Methyl Malonic Acidemia (3.4%), MSUD (3.3%) & Citrullinemia (2.8%). Interesting enough all these 5 common conditions are detectable by expanded new born screening. Of these 25 common disorders seen in Indian NICUs, we can detect atleast 33.88% cases (246/726) by simple expanded new bornscreening. Many of these disorders are treatable if detected early.

Table # 2 : Most common 25 Disorders in our cohort

Sr. No.	Disorder	Cases
1	NKHG	37
2	Galactosemia	29
3	Methyl Malonic Acidemia	25
4	MSUD	24
5	Citrullinemia	21
6	Camitine Transporter defect	20
7	Severe Biotinidase deficiency	19
8	Propionic acidemia	17
9	Respiratory Chain Defect	16
10	GA Type 11	11
11	Tyrosinemia Type 1	8
12	Cong Adrenal Hyperplasia	8
13	OTC Deficiency	8
14	Iso Valeric Acid demia	7
15	CPT II CACT Deficiency	7
16	Fructose 1,6 Diphosphatase Deficiency	6
17	Cystic Fibrosis	5
18	HIHA (Hyperinsulinism / Hyperammonemia)	4
19	HMG Co A Lyase Deficiency	4
20	3-Methyl Glutaconic Aciduria	4
21	G6PDeficiency	4
22	Pyridoxin Dependent seizures / Folinic Acid Responsive seizures(Antiquitin Gene Defect)	4
23	Neurotransitter metalic defect (SSADH and AADC-2 each)	4
24	Argininemia	4
25	Smith Lemli Opitz Syndrome	3
	Mitochodriopathy	79

Conclusion :- NKHG, Galactosemia, MMA, MSUD and Citrullinemia are 5 commonest IEMs in Critically ill newborns. 33.88 % of IEMs detected in NICU babies can be easily screened using Expanded Newborn Screening

Further readings :

1. Barbara K. Burton, MD, Inborn Errors of Metabolism: The clinical diagnosis in early infancy, Pediatrics, Vol. 79 No. 3, March 1987 : Pg 359 - 369.
2. Clark J. T. R., A clinical Guide to inherited Metabolic Diseases, Cambridge University press, 1996 : pg 176 – 204..

Need to monitor Oxidative stress by GSH in Critically Ill New Borns with Inborn Errors of Metabolism.

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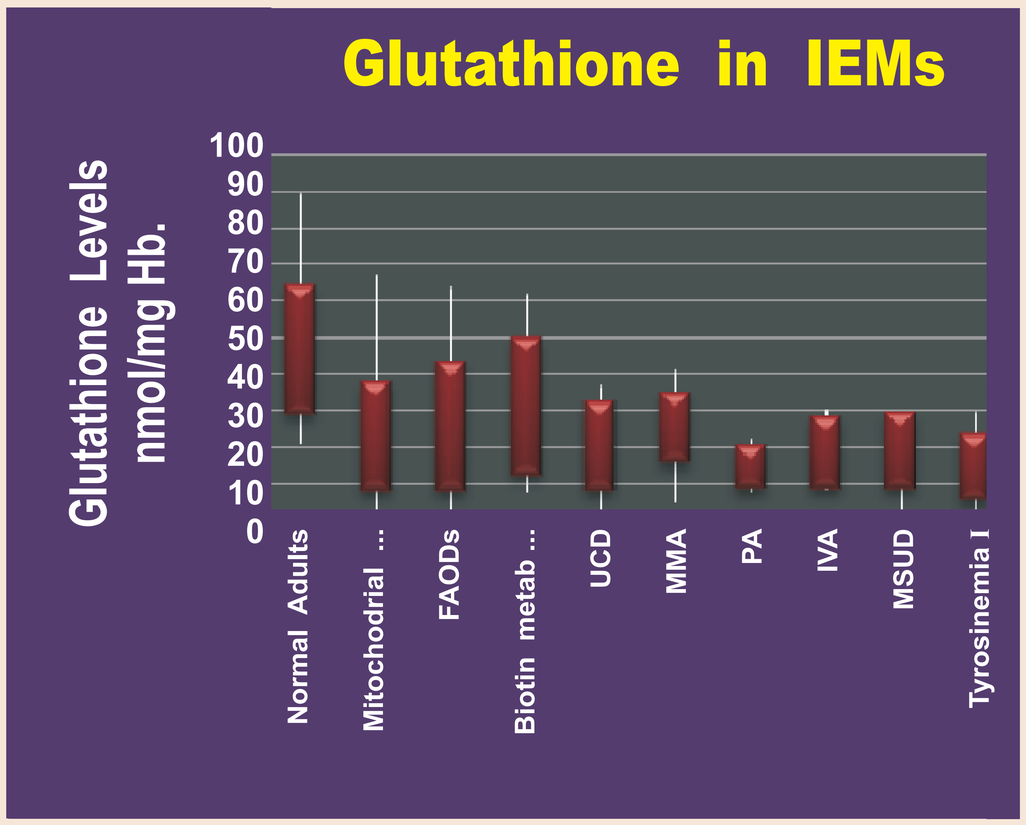
Introduction : Oxidative stress has been proposed as an important pathogenic feature in various Inborn Errors of Metabolism including mitochondrial disorders organic acidurias, amino acidemias and urea cycle defects.In times of metabolic crisis in these disorders the production of reactive oxidative metabolites is highly increased which could lead to rapid depletion of cellular anti - oxidant stores[1]. In this study, we demonstrate low levels of cellular anti-oxidant glutathione as an indicator of increased oxidative stress in critically ill new borns with Inborn Errors of Metabolism.

Subjects and Methods : To evaluate the redox status in neonates with IEMs we measured the levels of the cellular anti - oxidant, Glutathione as an indirect marker for oxidative stress. Study population consisted of neonates diagnosed to have various IEMs, such as mitochondriopathies, organic acidurias and amino acidemias. Glutathione levels in these patients were compared with those in healthy controls. Blood Glutathione analysis was performed by colorimetric enzyme recycling method.

Results : Significantly lower levels of the anti - oxidant, Glutathione were observed in new borns with inborn errors of metabolism.

Groups	GSH levels nmol/mg Hb.
Normal Adults (n=54)	46.64 + 17.51
Mitochondrial disorder (n=27)	22.85 + 14.75
FAODs (n=9)	25.37 + 17.49
Biotin metabolism disorder (n=8)	30.96 + 18.71
UCD (n=19)	20.27 + 12.16
MMA (n=19)	25.29 + 9.44
PA (n=4)	14.14 + 5.97
IVA (N=4)	18.33 + 9.84
MSUD (n=6)	19.01 + 10.38
Tyrosinemia (n=6)	14.35 + 9.13

Discussion : Oxidative stress plays an important role in the pathogenesis of many diseases including cancer, inflammation, diabetes, stroke, heart disorders, etc. Disorders affecting mitochondria, respiratory chain function or abnormalities in branched chain amino acid metabolism have been shown to be associated with impaired redox balance[1]. Increase in generation of intracellular oxidants has also been documented in many organic acidemias[2]. Our study demonstrates low levels of cellular anti - oxidant glutathione in patients with mitochondrial disorders, organic acidurias and amino acidemias. Low levels of this intracellular anti - oxidant indicate that these patients suffer from systemic oxidative stress and this redox imbalance may provide an important contribution to the disease pathogenesis. Furthermore, anti - oxidant therapies that improve redox balance may be beneficial for the cognitive and neurological outcome in these disorders, thus making monitoring of oxidative stress levels in these disorders very essential for the overall outcome.



Conclusions :- The significantly low levels of Glutathione in neonates with various Inborn Errors of Metabolism, indicates a relatively high level of Oxidative stress in these patients .This stress could in turn affect the pathogenesis and progression of the disease, thus making it essential to monitor oxidative stress in these conditions along with the other metabolic markers of the disease. Anti - oxidant supplementation in these patients may hence be recommended as a complimentary therapy to relieve the chronic oxidative

References : -

1. Atkuri K R, Cowam TM, et al. Inherited disorders affecting mitochondrial function are associated with glutathione deficienc and hypocitrullineemia. 2009, PNAS ; 106(10) : 3941-5.

2. Wajner M, Latini A, et al. The role of oxidative damage in neuropathology of organic acidurias : insights from animal studies. 2004, JIMD; 27 : 427 - 4

Usefulness of Plasma MMA level estimation in Methyl Malonic Acidemia in Newborns

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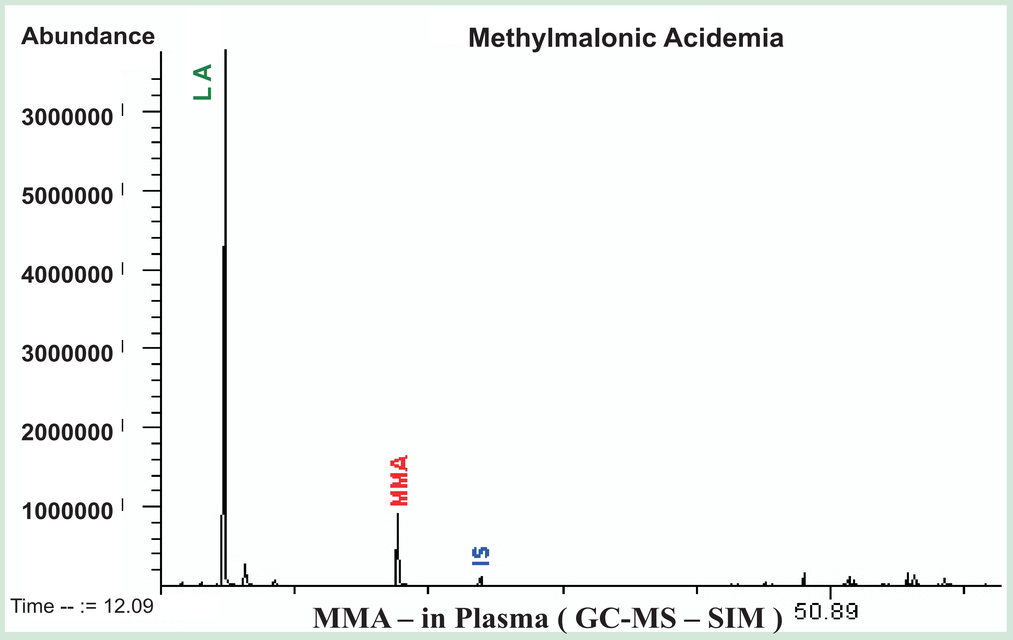
Introduction : - Methyl Malonic Acidemia (MMA) is a group of Organic acidemia, all being inherited in autosomal recessive manner and characterized by elevation of Methyl Malonic acid in urine and plasma. It is a group with many disorders – Mut^0, Mut^-, Cbl A, B, C, D (1). Identification of these specific types requires complementation studies (on cultured skin fibroblasts) or mutation studies by gene sequencing. However these studies are very costly, time consuming and mostly not available in developing countries like India. Hence a clinician has to rely on some simple measure s to understand the severity of MMA. Though we do not know exact incidence of MMA in our country, from our own data we have 23 MMA out of 665 critically ill newborns. However this data is of babies 0 - 90 days age. MMA can present later on also. It is clinically very difficult to predict out come based on basic parameters like Ammonia, Lactate or anion gap.However in our experience actual MMA level in plasma gives us a fairly good idea of the possible outcome.

Clinical features in a neonate :- Refusal to feed, Lethargy, Vomiting, Altered sensorium, Convulsions, Metabolic acidosis / Ketosis , Hypo / Hyperglycemia, Leucopenia / Leucocytosis, Thrombo - cytopenia, Elevated MMA in Urine and Plasma,Elevated ammonia, lactate, increased anion Gap.

Material and Method : - We analysed 7 cases of MMA detected in last 1.5 years (since we acquired our own GC - MS and started performing quantitative MMA estimation in urine and Plasma). Diagnosis was established on GC-MS of urine and TMS (Tandem Mass Spectrometry) of blood.Those cases which showed elevated MMA, methyl citrate and / or Tiglyl glycine in urine and elevated C3 on TMS were included in this study. We also measured Urine and Plasma MMA using SIM Mode technology along with Internal Standard to quantitate MMA level. Our experience showed that urinary MMA levels fluctuated quite a lot, especially due to varying Creatinine levels and food intake; therefore we preferred Plasma MMA levels for understanding the severity.

Results : - Normal Plasma MMA levels were found to be < 1.0 umol/L, though literature suggests < 0.376 umol/L (2). Lower levels (3.39 umol/L) had good outcome, where levels of 2,000 umol/L were associated with chronic renal failure. Baby VY expired despite not very high MMA level as parents decided not to treat this child. Child with > 2,000 umol/L of MMA received excellent treatment including special diets since very beginning in his life (D-3) and therefore has much better utcome. Those who survived with neurological damage have MMA of 8.41 and 440.2 umol/L. However these babies still do not have any evidence of renal failure.



Conclusion : - Though higher MMA levels seem to be associated with high mortality and morbidity, yet final outcome also depends upon proper dietary and medical treatment. Renal failure on the contrary seems to be associated with very high plasma levels and not usually seen with levels < 440 uMol/L in this cohort. Please note all the MMA patients in this cohort presented in the newborn period with recognizable signs and symptoms. Plasma MMA level estimation in the neonatal period will help in understanding severity of the disorder, prognostication and also help in treatment monitoring.

Ref. :

1. JTR Clark, metabolic Acidosis, A clinical Guide to inherited Metabolic Diseases, 2nd Ed., 93 – 95
2. CDC Website http://www.cdc.gov/nbbddd/b12/detection.html

Sr.	Name	Outcome	Age	Sex	Onset	S/S	Pl - MMA <1umol/L	Ur MMA <5umol/ mmol Cr
1	MD	Neurological damage	15 M	F	NB	Recurrent vomting, seizure, Metabolic Acidosis, Failure to thrive, Mentally retarded	4402	4802.91
2	A	Exp	15 M	F	NB	Hyperglycemia, Hemiparesis, Metabolic Acidosis	491	4151
3	VY	Exp	10 D	M	D - 1	Altered sensorium, Vomiting, Lethargy, Convulsions.	118.26	280.9
4	AB	Cardiomyopathy	2 M	M	NB	Grunting, Tachypnea, Cyanosis, Poor Cardiac output, Cardiomyopathy on ECHO	7.8	262.19
5	KS	Normal	4 M	F	D - 4	Breathlessness, cough, lethargy, Severe M Acidosis, hypoglycemia	3.39	66.7
6	MP	Neurological damage	18 M	M	NB	Lethargy, Vomiting, Metabolic acidosis, Altered sensorioum, convulsions	87.41	29060
7	DP	Renal Failure	31 M	M	D - 3	Lethargy, Vomiting, Metabolic acidosis, Altered sensorioum, convulsions, convulsions, renal failure.	2043.78	2822.05

Estimation of Non Esterified Free Fatty Acid as a screening for IEM in critical new borns

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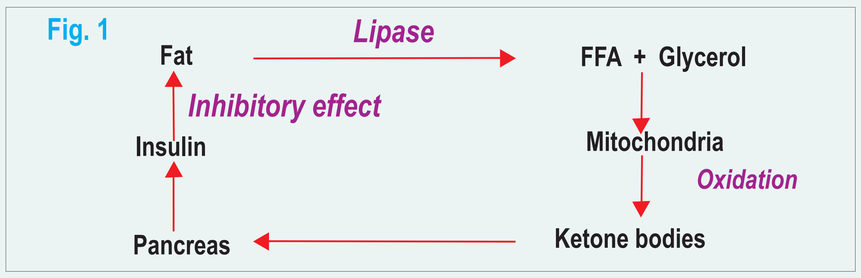
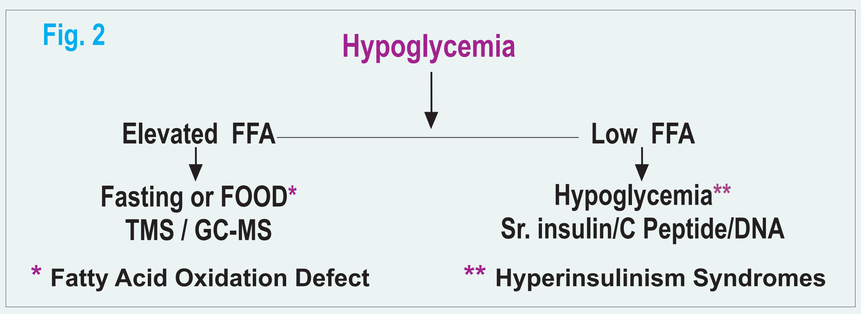
Introduction : - Non Esterified Free Fatty Acid (NEFA) in plasma is used as an important energy source for peripheral tissues. The amount of NEFA in plasma depends on a balance between intake in liver and peripheral tissues, and the release from adipose tissues. Lipolysis is a simple process whereby the fatty acids attached to glycerol (triacylglycerols) are hydrolytically removed yielding Free Fatty Acids (FFA) and glycerol. FFA serves as a precursor for the synthesis of ketone bodies (Figure 1). Increased levels of NEFA could be due to fasting, Fatty Acid Oxidation Defects (FAOD) or increase in levels of epinephrine whereas decreased levels may be because of hyperinsulinemia (Figure 2)[1]

Fig. 1



Levels of Free Fatty acid in plasma depend on following factors – Glucose, Insulin, Fasting, and Epinephrine

Fig. 2



Material and Method : - NEFA levels were established in children presented at our clinic with any of the following features – seizures, developmental delay and / or ketosis (< 18 yrs) as a part of their metabolic investigations[2] (n=36) (control group). Children with Hypoglycemia, FAOD and Hyperinsulinemia were excluded from this cohort. 6 children with Carnitine Transporter Defect, 2 with LCHAD (Long Chain Acyl CoA Dehydrogenase Deficiency), 1 with Hyperinsul - inemia and 2 with defects in ketone metabolism were included in the study group (n=35). Estimation of NEFA was performed using a standard kit by Wako[3].

Results : -

a] Control Group : - Reference range of 414 ± 174 (Mean ± 1SD) (240 – 588) uEq/L was established.

b] Study Group : - NEFA levels of this group were analysed, 6 with Carnitine Transporter Defect were found to have mean NEFA level of 879 ± 608 uEq/L, 2 with LCHAD had mean NEFA level of 1,365 ± 1,506 uEq/L, one with Hyperinsulinemic Hypoglycemia with level of 254 uEq/L and 2 with defect in ketone metabolism with mean level of 2,730 ± 300 uEq/L. Out of 6 children with Carnitine Transporter defects, 4 were < 30 days. 1 baby with LCHAD was < 30 days. It is a routine practice in NICUs to provide 5 - 10 % dextrose continuously to critically ill babies with hypoglycemia, and therefore it is possible that some individuals may show NEFA levels surprisingly low despite having FAOD (as seen in PL – case # 6).

Conclusion : - Free Fatty Acid is a good marker of Glucose metabolism and estimation of NEFA in Critically Ill New Borns (CINB) gives a good insight into Glucose and fat metabolism. As it is dependant on Glucose, Insulin, and Epinephrine, interplay of all these factors in a sick baby will ultimately result in either elevation or reduction of NEFA level. A regular estimation of NEFA in a sick newborn, especially those associated with Hypoglycemia can immediately alert a clinician about either a possibility of FAOD, Ketolysis defect or Hyperinsulinism, confirmation of which may be time consuming and costly. Thus from our experience we conclude that NEFA screening of any CINB will help in the diagnosis and management and should be adopted as a routine practice.

References : -

1. Dole VP. A relation between non esterified fatty acids in plasma and the metabolism of glucose. J Clin Invest. 1956 ; 35 (2) : 150 - 154.

2. Zschocke J, Hoffmann G. Vademecum Metabolicum – Manual of Metabolic Paediatrics. 2nd Ed. 6 – 7.

3. Shimizu S, Yasui K et al. Biochem. Biophys. Res. Commun. 1979 ; 91: 108-113.

Sr. #	Name	Age	Sex	NEFA	Free Carnitine	Diagnosis
1	OL	10 months	M	1,843	12	Carnitine Transporter Defect
2	AC	7 days	F	1,240	11.4	Carnitine Transporter Defect
3	PD	4 yrs	M	886	9.9	Carnitine Transporter Defect
4	RG	1 month	F	754	6.4	Carnitine Transporter Defect
5	A	6 days	F	430	14.9	Carnitine Transporter Defect
6	PL	1 month	F	123	8.09	Carnitine Transporter Defect
				879 ± 608 uEq/L		
7	IS	1 yr	F	2,430	12.3	LCHAD
8	HV	5 days	F	300	32.5	LCHAD
				1365 ± 1506 uEq/L		
9	VP	11 days	F	254	63.3	Hyperinsulinemic Hypoglycemia
				2730 ± 300 uEq/L		
10	NS	9 yrs	F	1,275	32.6	Ketolysis Defect
11	SN	1.5 yrs	F	1,699	75.8	Beta Ketothiolase Deficiency