Intracellular Glutathione Status in Metabolic Encephalopathies

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Introduction: - Over last 125 years we have seen immense progress in understanding of biochemistry and nutritional science of Glutathione and have witnessed the evolution of novel roles of GSH in signal transduction, gene expression, apoptosis, protein-glutathionylation & nitric-oxide metabolism[1].

Table 1: Roles of Glutathione

1.] Antioxidant defense:-
Scavenging free radicals and other reactive species
Removing hydrogen and lipid peroxides
Preventing oxidation of biomolecules

2.] Metabolism
Synthesis of leukotrines and prostaglandins
Conversion of formaldehyde to formate
Production of D- lactate from methyl glyoxal
Formation of mercapturates from electrophiles
Formation of Glutathione – NO adduct
Storage and transport of cysteine

3.] Regulation
Intracellular redox status
Signal transduction and gene expression
DNA and protein synthesis
Cell proliferation and apoptosis
Cytokine production and immune response
Protein glutathionylation
Mitochondrial function and integrity

Role of GSH as a neurotransmitter and neuromodulator in the CNS, with a complete **γ**-glutamyl cycle and specific GSH binding sites in the brain is evident[2]. Most of the cellular GSH (85-90 %) is present in the cytosol, with the remaining in organelles. It is oxidized non enzymatically to glutathione disulfide by electrophilic substances (free radicals and reactive oxygen / nitrogen species). The GSSG efflux from the cell contributes to the net loss of intracellular GSH, generally seen in protein malnutrition, oxidative stress etc.

GSH + 2GSSG = total cellular Glutathione

The GSH:GSSG ratio (>10 under normal conditions), the major redox couple, determines the antioxidative capacity of cells. But its value can be affected by other redox couples, including NADPH / $NADP^+$ and $thioredoxin_{red}$ / $thioredoxin_{ox}$.[1] There is a therapeutic possibility of modifying intracellular GSH status by supplementation of Vit C, Vit E and *N*-Acetyl Cysteine[2].

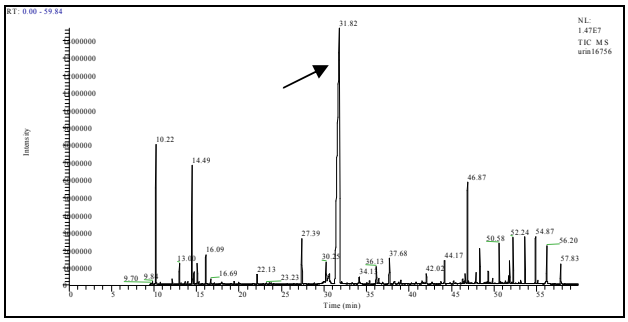
Aim and Objective: Since there are no specific signs or symptoms of reduced GSH levels either in newborns or older individuals, it is practically impossible to screen someone only clinically. Since oxidative stress is expected to be high in children suffering from various Inborn Errors of Metabolism especially Organic Acidemias and Mitochondriopathies, we made an attempt to identify specific groups of disorders more vulnerable to oxidative stress. Aim of the study was to determine intracellular reduced Glutathione levels (GSH) in children critically ill due to various inborn errors of metabolism.

Material and Method: 44 critically ill children with metabolic encephalopathy that presented with or had associated convulsions were screened for GSH assay over a period of 1.5 years [Jan 2008 – July 2009]. For GSH assay blood samples were collected in EDTA tube and immediately frozen at -20°C till assay. Hemolysate hemoglobin was estimated and biochemical colorimetric analysis was performed for the estimation of intracellular (RBC) GSH[3]. For identification of various IEMs, appropriate tests like GALT & Biotinidase enzymes, Aminoacids, TMS and GC-MS were employed as and when necessary.

Results:- Table # 1: Glutathione status in various IEMs.

	IEM	N=	Mean ± SD
1	Normal adults	9	52.79±64.10
2	Normal Children	19	55.25±62.31
3	Glutathione synthatase Defect	2	0±0
4	FAOD	5	15.71 ±15.58
5	Glycine Encephalopathies	7	24.64±52.96
6	MSUD	5	65.44±98.76
7	Organic Acidemias: MMA / GA	8	86.38±76.14
8	Mitochondriopathies	12	66.49±114.81
9	Peroxisomal Disorders	1	35.373
10	Biotinidase deficiency	3	30.64±30.43
11	OTC Deficiency	1	49.08

Fig. 1: Large peak of 5 Oxoproline at RT 31.82



Discussion: - Significantly low values of GSH were seen in Glutathione Synthatase defect (2), Fatty Acid Oxidation defects (5) & Glycine-encephalopathy (7). Typically Glutathione Synthatase defect children showed 5 Oxoprolinuria (Fig.1) along with metabolic acidosis and anemia. These two children with zero activity of GSH were treated with Vit C, Vit E and N-Acetylcysteine and both showed excellent recovery. Other disorders did not show any significant reduction of GSH in this cohort.

Conclusion: - Estimation of intracellular GSH activity may help in therapeutic management and better clinical outcome, especially in those who have associated metabolic acidosis and anemia.

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References:

1. Wu G, Fang YZ, Yang S, et al., Glutathione Metabolism and its Implications for Health. Recent Advances in Nutritional sciences (2004) 489-492.
2. Ristoff E, Larsson A, Oxidative Stress in Inborn Errors of metabolism, JIMD, 25 (2002), 223 – 226.
3. Eyer P. and Podhradsky D., Evaluation of micro method for determination of Glutathione using enzymatic cycling and Ellman's reagent. Anal. Biochem. 153, (1986) 57-66.

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