Monitoring of reduced Glutathione in Indian children with Homocystinuria and response to treatment

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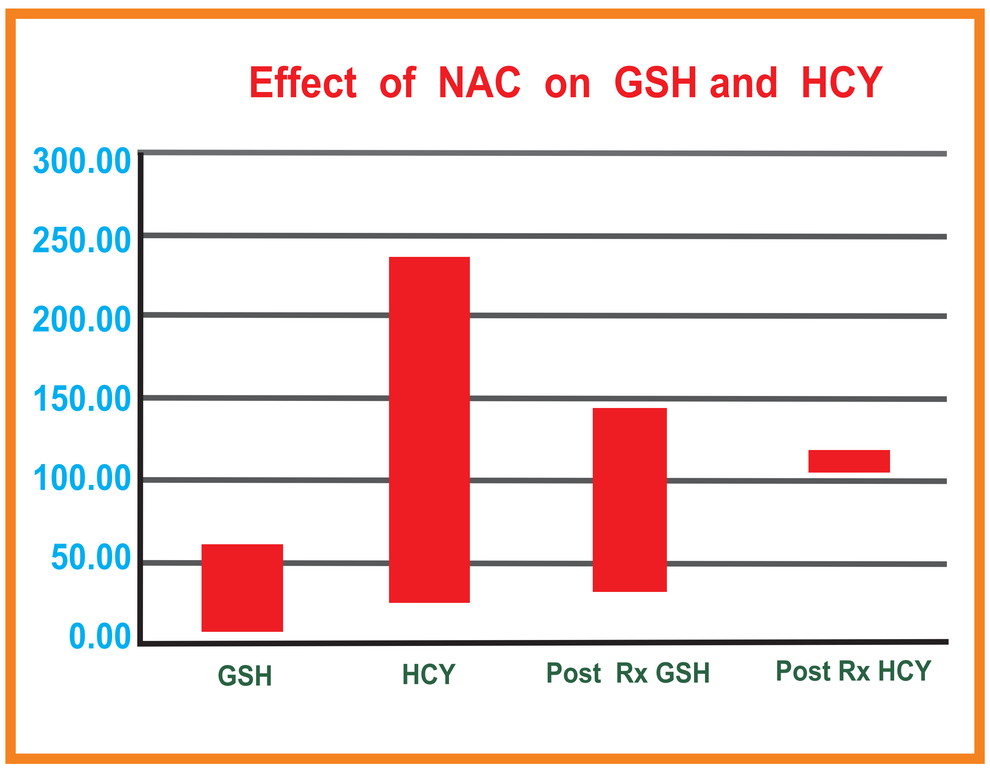
Introduction : Homocystinuria is a rare autosomal recessively inherited metabolic disorder. Classical Homocystinuria caused by mutations in the cystathione β- synthase (CBS) gene is associated with elevations of plasma and tissue homocysteine and methionine, which lead to a range of vascular and neurological sequelae and multiple connective tissue disturbances[1].

Objective : To analyse the levels of glutathione as a measure of oxidative stress in patients with homocystinuria and to determine the relationship between pathological levels of homocysteine and methionine in these patients with oxidative stress in response to medication.

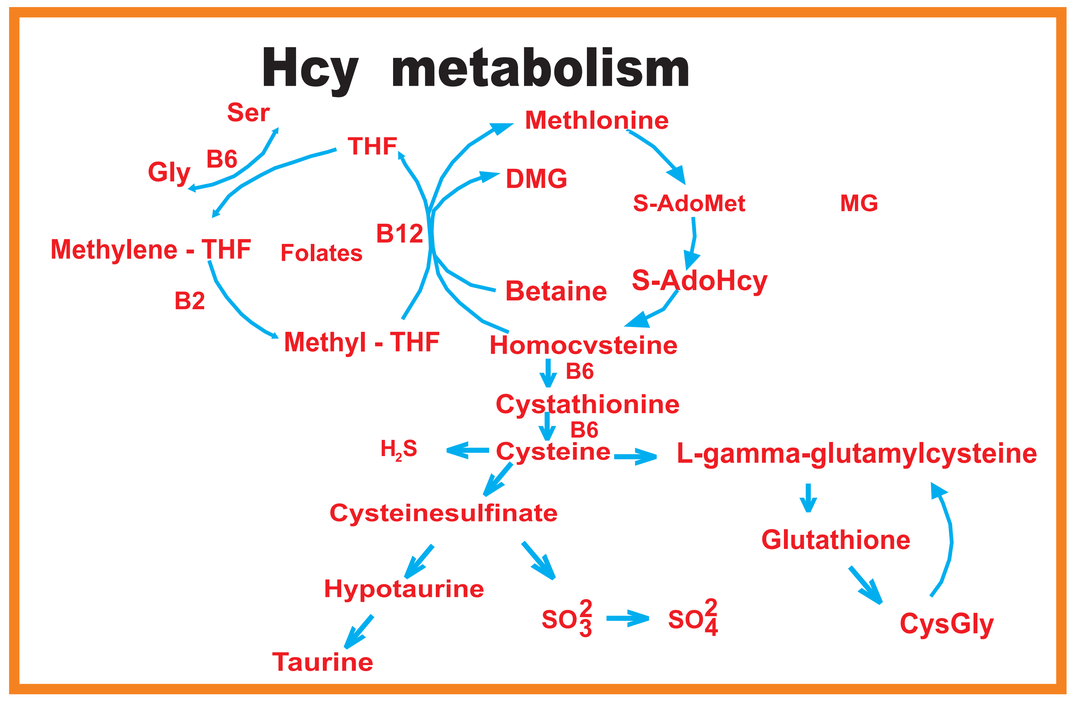
Materials & Methods : We analysed glutathione levels in over 600 patients with suspected IEMs. Out of these 4 patients were confirmed to have homocystinuria. These patients were followed up on a regular basis for their glutathione levels along with other parameters such as homocysteine, methionine, folic acid, vitamin B_{12}, calcium, phosphorus, alkaline phosphatase, total proteins and albumin. Blood samples of 54 healthy individuals were also analysed for reference ranges of glutathione in normal population.

Results : We found considerably reduced levels of glutathione in patients with homocystinuria (27.32 ± 19.65nmol/mg Hb, n=4) as compared to healthy individuals (46.64 ± 17.51 nmol/mg Hb, n=54). Glutathione levels were found to vary inversely with levels of homocysteine and methionine indicating increased oxidative stress with increasing levels of homocysteine and methionine. Significant improvement of Glutathione levels and reduction of homocysteine and methionine levels was observed upon adding N- acetyl cysteine supplements to the standard line of treatment with betaine, folate and cobalamin.

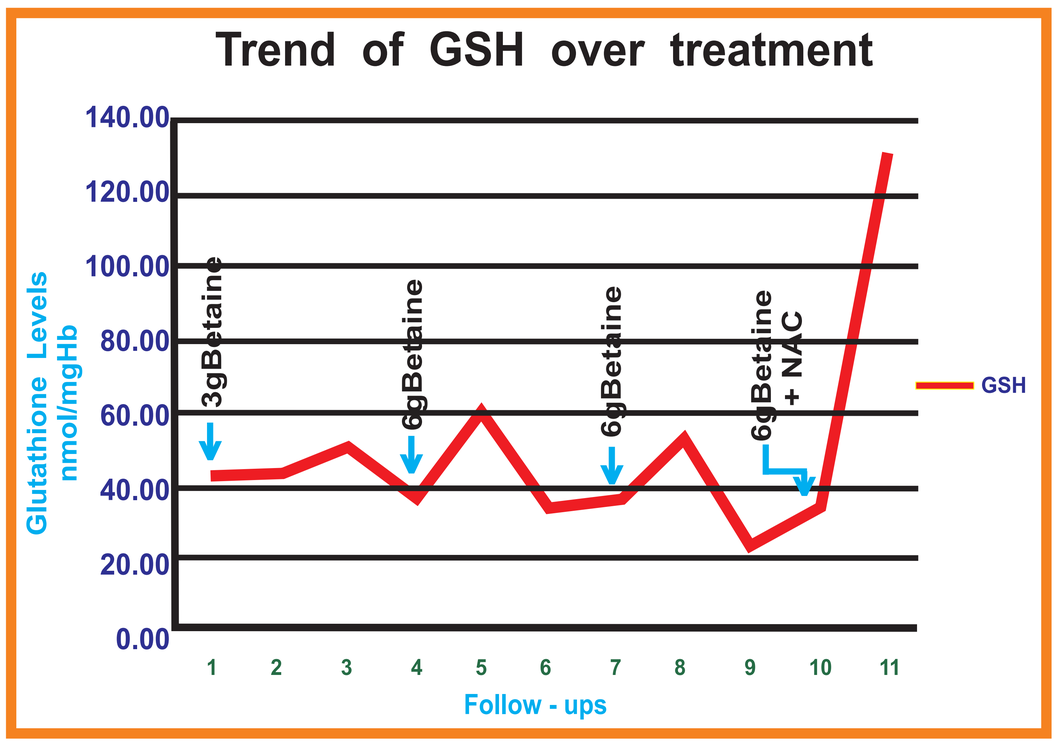


Discussion : Cystathione β- synthase is responsible for trans - sulfuration of homocysteine using pyridoxine as a co - factor and its deficiency therefore results in a trans - sulfuration defect with increased levels of homocysteine and methionine. Homocystinuria has detrimental effects on numerous organs and systems, and if untreated the condition can be fatal[1]. It is a treatable condition and early treatment can prevent development of ocular, skeletal and thrombo - embolic complications and promote development of normal intellectual capacity. Treatment options include betaine alone or in combination with pyridoxine, folate, cobalamin and methionine - restricted diet[2].

We studied 4 patients presenting to us with homocystinuria. Three patients out of these presented to us with myopia, lens dislocation, but normal mental and motor milestones. The mean age of presentation was 4 yrs. One patient (patient S) was detected by new born screening and has been on treatment since first week of life. These patients are followed up on a regular basis and various clinical and biochemical parameters are monitored including homocysteine, methionine, vitamin B_{12} and glutathione. The mean follow - up homocysteine levels of patients detected late in life were significantly higher (250.2 ± 102 umol/L) than those of the patient S detected by new born screening (28.5 ± 2.5 umol/L) suggesting chronic hyper homocysteinemia in the patients detected late. These patients now have complaints of poor scholastic performance, behavioural abnormalities and hyperactivity



We analysed glutathione levels in these patients and found considerably low levels of reduced glutathione (27.32 ± 19.65nmol/mg Hb, n=4) as compared to healthy individuals (46.64 ± 17.51 nmol/mg Hb, n=54). Patient S showed glutathione levels of 37.67 ± 24.47 nmol/mg Hb. We found glutathione levels to vary inversely with homocysteine and methionine, indicating oxidative stress with increasing levels of these metabolites. A significant increase in glutathione along with decrease in levels of homocysteine and methionine was observed upon adding N - acetyl cysteine to the standard treatment of betaine, pyridoxine and folate. The clinical improvement in these patients however needs to be evaluated over a longer period of treatment and follow- up.



Chronic hyper homocysteinemia as observed in these patients causes oxidative damage resulting in vascular manifestations such as myocardial infarction, cerebral thrombosis, hepatic steatosis, and pulmonary embolism[3]. Conventional treatment of CBS deficiency by diet and betaine tends to normalize many but not all metabolic abnormalities associated with CBS deficiency. Addition of anti - oxidants like N - acetyl cysteine and vitamin E showed better biochemical response in our cohort

Conclusion : Reduced Glutathione level monitoring and Anti - oxidant supplementation has shown beneficial effects in patients with Homocystinuria and merits further exploration. The clinical consequence of this need to be evaluated over a longer period.

References :

1. Mudd SH, Skovby F, Levy HL. The natural history of Homocystinuria due to cystathione β - synthase deficiency. Am J Hum Genet. 1985; 37: 1-31.
2. Wilcken DE, Wilcken B. The natural history of vascular disease in homocystinuria and effects of treatment. J Inher Metab Dis. 1997; 20 : 295 - 300.
3. dA Cunnha AA, Farreria AG, dA Cunnha MJ, Pederzolli CD, Becker DL, Coellho JG, Dutra- Filho CS, Wyse AT. Chronic hyperhomocystinemia induces oxidative damage in rat lung. Mol Cell Biochem. 2011; 385 (1-2): 153 - 160.

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