Status of Glutathione in Indian children with Citrullinemia type I

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Introduction : Citrullinemia (OMIM#251700) is an autosomal recessive disorder caused by deficiency of enzyme argininosuccinate synthase (ASS). It is characterized by accumulation of ammonia, citrulline and orotic acid in blood and other body fluids. The incidence world wide of this disease is 1:57,000[1]. However Indian incidence is not known.

Objective : To analyze the levels of glutathione as a measure of oxidative stress in patients with citrullinemia and to determine the relationship between pathological levels of ammonia and citrulline in these patients with oxidative stress.

Materials & Methods : We analysed glutathione levels in 600 patients with suspected IEMs. Out of these 15 patients were confirmed to have citrullinemia. Of these 11 patients succumbed to death in the early neonatal period, 4 survived and are being followed up on a regular basis for their glutathione levels along with other parameters such as citrulline, ammonia, orotic acid, 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. Peripheral blood samples of the subjects were collected in EDTA vacuutainers. The erythrocytes were separated and hemolysed immediately and the protein fraction were precipitated with meta-phosphoric acid. The supernatants were stored at -20°C until analysis. Biochemical colorimetric analysis for quantification of GSH was performed by enzymatic recycling method, using Glutathione reductase and Ellman's reagent[2].

Results : We found considerably low levels of glutathione in patients with citrullinemia (24.42 ± 14.98 nmol/mg Hb, n=15) as compared to healthy individuals (46.64 ± 17.51 nmol/mg Hb, n=54). The patients who succumbed in the neonatal period showed even lower levels than others in the same group; (16.24 ± 13.33 nmol/mg Hb, n=11) Glutathione levels were found to vary inversely with levels of ammonia indicating increased oxidative stress with increasing levels of ammonia ($p<0.01$). Citrulline did not vary significantly with glutathione levels. The 4 patients who survived were followed up at regular intervals. Significant improvement in Glutathione levels upon controlling ammonia was noted.

Discussion : Citrullinemia type I usually becomes evident in the first few days of life. Affected infants typically appear normal at birth, but as ammonia builds up in the body, they develop lethargy, poor feeding, vomiting, seizures and loss of consciousness. These can be life -threatening in many cases. Mutations in the ASS gene result in deficient activity of the enzyme Argininosuccinate synthase (ASS) (EC 6.3.4.5), which disrupts the urea cycle and prevents the body from processing nitrogen effectively. This results in accumulation of excess nitrogen in the form of ammonia, and other by-products of urea cycle in the blood stream.

It is a treatable condition and early treatment can prevent death and mental retardation. Treatment includes controlling ammonia, protein restricted diet and arginine.

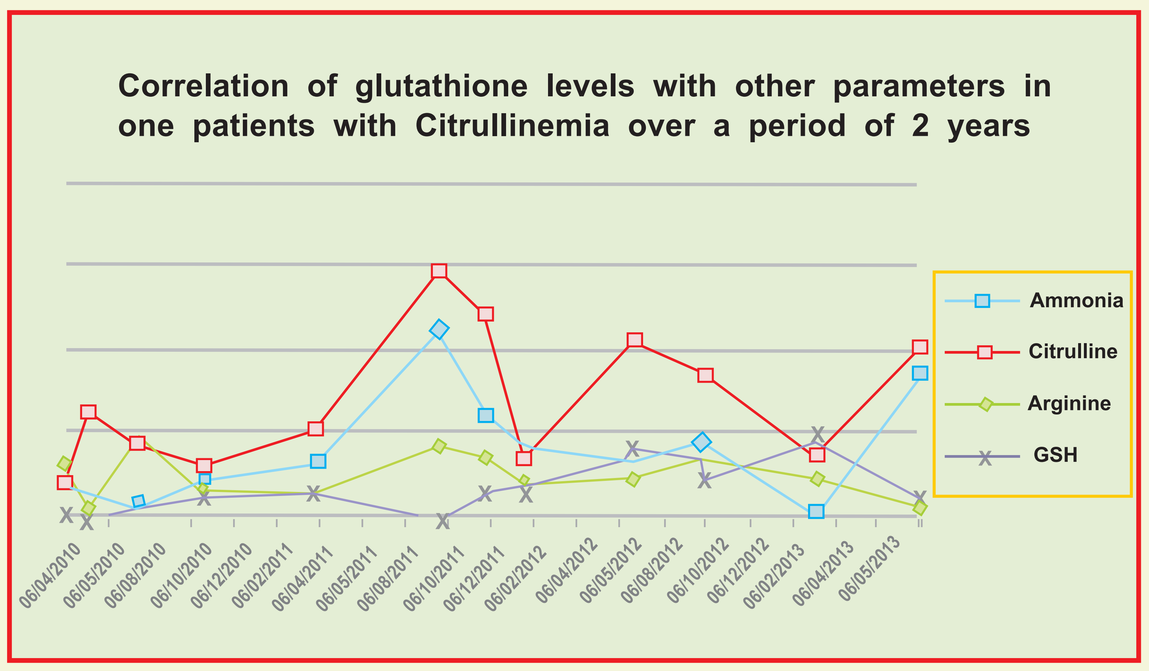
We studied 15 patients with citrullinemia. These patients presented with lethargy, vomiting, convulsions, refusal to feed in the early neonatal life. 4 out of these survived, while the rest succumbed to the life threatening episodes during neonatal period. The surviving patients are being followed up on a regular basis and various clinical and biochemical parameters are monitored including citrulline, ammonia and glutathione.

We analysed glutathione levels in these patients and found considerably low levels of reduced glutathione (24.42 ± 14.98 nmol/mg Hb, n=15) during the first presentation as compared to healthy individuals (46.64 ± 17.51 nmol/mg Hb, n=54).

Glutthione levels in the patients who could not survive the neonatal period were lower than the others in the group; (16.24 ± 13.33 nmol/mg Hb, n=11) ($p<0.01$) indicating that oxidative stress may play an important role in the survival of these patients. Lower levels of glutathione imply greater degree of oxidative stress in these patients at the time of metabolic crisis.

Patient Groups	GSH Levels (mean ± SD) (nmol/mg Hb)
Expired patients with Citrullinemia (n=11)	16.24 ± 13.33
Alive patients with Citrullinemia (n=4)	24.42 ± 14.98
Normal adults (n= 54)	46.64 ± 17.51
P Values (Mann Whitney U test) (Expired v/s Normal)	< 0.01
P Values (Mann Whitney U test) (Surviving v/s Normal)	< 0.05

The 4 patients who survived the neonatal period were followed up on a regular basis. We found glutathione levels to vary inversely with ammonia, indicating oxidative stress with increasing levels of this metabolite. A significant increase in glutathione was observed upon improvement of ammonia status in these patients. Intercurrent metabolic crisis led to decrease in levels of glutathione indicating increased oxidative stress during this period. We hypothesize that supplementation with anti- oxidants during intercurrent illness may improve the survival chances in these patients and may also prevent brain damage due to excessive accumulation of oxidative metabolites.



Conclusion : Low levels of glutathione were observed in patients with Citrullinemia. Better levels of glutathione within the group seemed to improve the chances of survival in the time of intercurrent illness. Glutathione levels correlated inversely with levels of ammonia indicating oxidative stress in these patients during intercurrent illness. The clinical consequence of this observation merits further investigation and needs to be evaluated over a longer period.

Conflict of interest : None

References :

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