CLINICAL AND BIOCHEMICAL STUDIES IN HOMOCYSTINURIA

Manjeet Kaur
Madhulika Kabra
G.P. Das
M. Suri
I.C. Verma

ABSTRACT

Homocystinuria was diagnosed in 15 (0.59%) cases on screening 2560 children for aminoacidopathies. The commonest presenting features were ectopia lentis (95%) and mental retardation (86%). Other features included, dental anomalies (40%), osteoporosis (40%), behavioral problems (33%) and arachnodactyly (13%), Diagnosis was confirmed by iodoplatinate staining of one dimensional paper chromatography of urine.

All the 15 cases of homocystinuria were first treated with high dose oral pyridoxine. Only one case responded to pyridoxine therapy. All the other patients were started on a low methionine, High cysteine diet with folate supplementation. Only one patient showed a complete response to dietary therapy. Nonavailability and high cost of the commercially available methionine-free, cysteine-supplemented diet and late diagnosis were responsible for the poor response in the majority of our patients.

Key words: *Homocystinuria, Ectopia lentis, Mental retardation.*

From the Genetics Unit, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi 110 029.

Reprint requests: Professor I.C. Verma.

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Homocystinuria is an inborn error of sulphur containing aminoacids caused by a deficiency of cystathionine β-synthase. It was first discovered in 1962, during screening of mentally retarded individuals for aminoacid disorders(l). In India, Rao *et al.(2)* documented 3 (0.24%) cases of homocystinuria on screening mentally retarded subjects in Bombay, while Reddi *et al.* (4) found no case among 2100 patients screened in Hyderabad. From the Genetic unit, 4 cases in 2 families were published earlier(5,6). In the present study we report the clinical and biochemical profile of 15 cases of homocystinuria, diagnosed and treated over a period of 7 years.

Materials And Methods

Between January 1986 and December 1992 we screened 2560 patients, for amino acid disorders. These included patients with delayed milestones, mental retardation, intractable seizures, abnormal skin and body odor, ectopia lentis, *etc.* The patients were referred from the Pediatric Outpatient and the Eye Centre at AIIMS, and other Delhi hospitals and Nursing homes. All the patients were from North India.

Every patient was examined for clinical features of an inborn error of amino acid metaolism. A check list was used to look specifically for the various manifestations of homocystinuria. Initial screening for homocystinuria was done on samples of urine by cyanidenitroprusside and silver nitroprusside tests(7). The diagnosis was confirmed by one dimensional paper chromatography of urine and plasma(7). To avoid binding of homocysteine to plasma proteins; blood samples were centrifuged and plasma deproteinized with sulphosa-

licylic acid within 30 minutes of collection. Briefly, aliquots of urine containing 16 ug of creatinine, or 10 µl of deproteinized plasma, were applied on Whatman 3 MM chromatography paper. Chromatograms were developed overnight (ascending) in a freshly prepared solvent mixture consisting of n-butanol, glacial acetic acid and water in 12:3:5 ratio. Amino acids were located by dipping the chromatograms in iodoplatinate reagent(8). Homocysteine, methionine and mixed disulfide of homocysteine and cystine were observed in plasma and urine of these patients *(Fig. 1).*

After confirmation of diagnosis, all patients were given a therapeutic trial of pyridoxine using an initial dose of 120-300 mg/d depending on the age. If no beneficial effects were observed, the dose was slowly increased to 1200 mg/d in divided doses. Biochemical response to pyridoxine therapy was monitored by chemical tests on urine and chromatography of plasma for methionine and homocysteine. If the patients urine and plasma methionine and homocysteine levels normalized with pyridoxine alone, it was continued indefinitely in the same dose. If there was no or a partial response, the patients were started on a low methionine and high cystine diet with folic acid supplementation (1-5 mg/d). Response to therapy was assessed by improvement in behavior, and tests for hompcysteine in the urine. Development of complications was also evaluated on follow up.

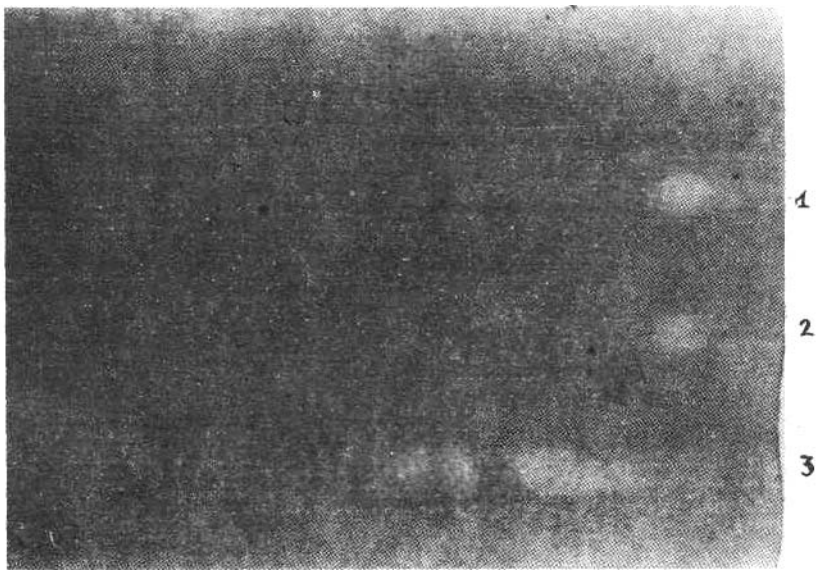

Fig. 1. *Showing spots of homocysteine, methionine and mixed disulfide of homocysteine and cysteine on chromatogram of urine, stained by iodoplatinate reagent.*

Results

Of the 25.60 children screened for disorders of amino acid metabolism, 15 (0.59%) cases were detected to have homocystinuria. Fifty four patients were referred with bilateral nontraumatic ectopia lentis, of whom 13 (24%) had homocystinuria. Details of the other amino acid disorders detected during this study are published elsewhere(9).

The diagnosis of homocystinuria was confirmed in 12 cases by urine and plasma chromatography. In 10 cases significant hypermethioninemia was present, whereas 1 case had slightly raised methionine levels in plasma. Enzyme assay was not done. In 3 cases diagnosis was based on urine chemical tests and aminoacidogram, since the patients did not allow blood sampling.

There were 9 boys and 6 girls with a sex ratio of 3:2. The mean age at presentation was 8.4 years. Ten patients were from Punjab, three from Uttar Pradesh and one each from Bihar and Madhya Pradesh. Parental consanguinity was present only in 2 (13%) patients.

Tables I and *II* give the clinial data of the patients. The two commonest presenting features were ectopia lentis and mental retardation. Of the 13 patients with mental retardation, 7 had severe and 6 had mild retardation. Surprisingly, short stature was more frequently seen than tall stature in our patients. No case presented with an evident thromboembolic event. The mean follow up was 14.7 months with a maximum of 4 years.

Response to Treatment

One patient (Case 15) showed complete response to pyridoxine. This girl presented with bilateral subluxation of lenses. Three months after being started on pyridoxine (120 mg/d) her urine aminoacidogram showed a marked decrease in homocysteine excretion and her plasma methionine level as normal. Same dose of pyridoxine was continued with a normal diet, and her urine tests have remained negative during follow-up. She is of average intelligence.

In another patient (Case 12), a higher dose of pyridoxine (1200 mg/day) produced a partial response. This child was subsequently started on a low methionine, high cystine diet with folate supplementation, while the high dose pyridoxine was continued. However, the child did not adhere to the strict dietary regimen and was lost to follow-up.

Only 1 child (Case 11), showed a good response to strict dietary control. This girl was unresponsive to pyridoxine therapy. She was started on a special methionine-free commercial formula, containing a balanced mixture of other essential and non-essential amino acids (supplemented with cysteine), carbohydrates, minerals, trace elements and vitamins (Maxamaid RVHB, Scientific Hospital Supplies Ltd, U.K). She was also supplemented with folic acid (5 mg/day). Both her eyes have been operated for ectopia lentis, and she is doing well at school. The other cases could not be started on Maxamaid RVHB because of its nonavailability and the high cost (Rs 600 per Kg), if imported. Some of the patients showed a partial response to a methionine-restricted diet *(Appendix A and B)*. However, all the patients found it difficult to maintain this restricted diet.

Discussion

Our data shows that homocystinuria

TABLE I—*Clinical Details of 15 Patients with Homocystinuria*

Case No.	Age at diagnosis (yr)	Sex	Clinical features	Response	Follow up
1.	10.5	F	EL, MR, LH, A, CT, PC	No	3 mo
2.	4	M	EL, MR	No	3 mo
3.	6	M	EL, MR, O, AR, BP	No	6 mo
4.	9	M	EL, MR, A, O, G, GV PC, BP	Partial response to diet	3 year
5.	11	F	EL, TS, MR, LH, C, Wt<5th O, AR	?	1 mo
6.	10	F	SS, EL, MR, G, O, PC,	No	2 mo
7.	7	M	EL, MR, CO, LH, BP, DM	No	2 year
8.	12	M	MR, SS, O, DM, Wt<5th, AR	?	1 mo
9.	8	F	EL, MR, M, DM, Wt<5th SS, AR, DM	?	1 mo
10.	16	M	EL, MR, SS, DM, AR Wt<5th, BP	No	2 mo
11.	4	F	EL, AR, TS	Response to diet	4 yr
12.	6	M	EL, MR, M, TS, DM, PC, GV, O, AR	Partial response to B_6	6 mo
13.	7	M	EL, MR, SS, Wt<5th, DM, BP	No	3 mo
14.	6	M	EL, MR, SS, DM, Wt<5th AR	?	1 mo
15.	10	F	EL, AR, TS	Response to B_6	3 yr

EL-Ectopia lentis, MR-Mental retardation, LH-Light hair, A-Arachnodacyly, O-Osteoporosis, CT-Carious teeth, G-Glaucoma, PC-Pectus carinatum, AR-Advanced ratio, BP-Behavioral problems, TS-Tall stature, SS-Short stature, Wt<5th-centile-ICMR Standard, C-Cataract, GV- Genu valgum, DM- Dental malocclusion, M- Myopia, Co-Corneal opacity, DF-Dysmorphic face.

is a common inborn error of amino acid metabolism in North Indian children. Although the prevalence of this disorder (0.59%) is higher than that reported from other parts of India(2-4), this may be due to the fact that the patients screened included cases not only with mental retardation but other presenting complaints as well, whereas the other studies had restricted their screening to patients with mental retardation only. Secondly cases were referred from the R.P. Centre of Ophthalmic Sciences which is a very large eye hospital. Similarly, figures from the West are also extremely variable. Carson *et al*.(1) found 10 cases

TABLE II–*Frequency of Clinical Features in Homocystinuria* (n=15)

Clinical features	Number	%
Ocular		
Ectopia lentis	14	95
Myopia	2	13.3
Glaucoma	2	13.3
Cataract	1	6.6
Corneal opacity	1	6.6
Skeletal		
Tall stature	4	26.7
Arachnodactyly	2	13.3
Short stature	6	40
Disproportionate stature (Advanced US/LS ratio)	9	60
Genu Valgum	2	13.3
Osteoporosis	6	40
Central nervous system		
Mental retardation	13	86.6
Behavior Problems	5	33.3
Miscellaneous		
Dental abnormalities	6	40
Fine, light, brittle hair	4	26.7

(0.34%) on screening 2920 cases of mental retardation in Northern Ireland, whereas in North America, homocystinuria had a prevalence of 0.02% among cases of mental retardation(10). Schmike *et al.* (11) estimated that in North America, 5% of patients with ectopia lentis are expected to have homocystinuria. In the present study we observed that 24% of the cases with non-*f* traumatic dislocation of lens had homocystinuria. This probably reflects a greater awareness about homocystinuria among the referring doctors.

All our patients had "classical" or type 1 homocystinuria since all of them, had hypermethioninemia. The patients of type 2 homocystinuria present early with vomiting, poor feeding, lethargy, hypotonia and developmental delay. These patients have homocystinuria, hypomethioninemia and megaloblastic anemia. Type 3 homocystinuria may present both as an acute neonatal illness or with chronic manifestations. These patients also have hypomethioninemia but do not have megaloblastic anemia(12,13).

The two commonest presenting features were eccopia lentis (95%) and mental retardation (86.6%). The frequency of these findings have been reported to vary from 55-100% and 84-100%, respectively in other studies(l,13). Tall stature and marfanoid habitus were infrequently seen in our patients. Instead, short stature or normal stature with advanced upper to lower segment ratio was seen more frequently. This may be due to the poor nutritional status of these patients as 4 out of 9 cases with advanced upper to lower segment ratio had weights and heights less than 5th centile for their age (ICMR standards).

Verma *et al.(6)* reported thromboembolic events in 2 brothers, who were 16 and 11-year-old, respectively. Mudd *et al.* (14) observed that thromboembolk disease is infrequent before the age "of 20 years. We did not have any case with an evident thromboembolk episode probably due to younger age of our patients and short duration of follow up. Many investigators suggest that hbmocystinuria should be considered in the differential diagnosis of venous or arterial thrombosis, even in "the absence of other manifestations of the disease, and regardless of age(15,16). Even the heterozygotes for hpmocystinuria have an increased predisposition to peripheral and cerebrovascular arterial disease. Hence, the detection of these cases is also of great importance(17,18).

Only one of our patients was pyridoxine responsive. This case presented at 10 years of age with normal intelligence. McKusick *et al.* (19) have also documented a mildly affected patient with homocystinuria due to cystathionine synthetase deficiency, who had normal intelligence and developed ectopia lentis after 10 years of age. Their patient also showed clinical and biochemical response to pyridoxine. Both pyridoxine responsive as well as unresponsive type of homocystinuria have been reported from the West(20,21). Recently, the molecular basis of cystathionine synthetase deficiency in pyridoxine responsive and unresponsive patients has been elucidated(22).

A low methionine and high cysteine diet helps in maintaining intellectual development. Introduction of dietary therapy is worthwhile at any age, but is very costly for the Indian patient, as the commercially produced low methionine and high cysteine formulas are not available and need to be imported. Therefore, most of the times, one has to plan a special diet for patients, using locally available foods. These diets aim to provide 25-45 mg/kg/d of methionine in infants and$_t$ 8-10 mg/kg/d of methionine in teenagers. Plasma levels of methionine are to be maintained between 0.03-0.1 mmol/1 and cystine levels between 0.037-0.085 mmol/l(23). Although, ideally, quantitative measurments of plasma methionine are required, the same information can be obtained by paper chromatography after careful standardization. *Appendix A* gives a list of restricted and unrestricted food items in homocystinuria. Since wheat and pulses are to be restricted, the caloric density of the diet can be increased by mixing sago or arrowroot powder in wheat flour. A prototype diet for a 10 kg child is shown in *Appendix B*. It is difficult to increase the cystine content of this diet without inadvertently increasing its methionine content. Therefore, these diets are not as effective as the commercial diets.

Treatment with folic acid (1-5 mg/d) is worthwhile in all the cases. Treatment with betaine (trimethylglycine; 6-9 g/d) which serves as a methyl group donor, produces clinical improvement in patients unresponsive to pyridoxine(23,24).

Homocystinuria is a distressing disorder as it leads to mental retardation and visual impairment. There is a 25% risk of recurrence in each pregnancy. Prenatal diagnosis is possible by measuring cystathionine synthetase activity in cultured cells from chorionic villus biopsy at 10-12 weeks or amniocentesis at 15-18 weeks of gestation(12). Therefore, early diagnosis is not only important for preventing visual and mental handicap in the proband, but also preventing the birth of another affected child. This may eventually help in reducing the burden of this disease in the community.

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APPENDIX *A—Diet For Homocystinuria Patient*

List A—Forbidden foods

(a) Meat, chicken, fish, eggs; *(b)* Milk, cheese, curd, ice-cream, chocolates, horlicks, ovaltine; (c) Wheat flour, bajra, maize, barley, jowar, oat meal, bread, cakes, biscuits and pasteries; *(d)* Rice and pulses; (e) Nuts and dried fruits; *if)* Maggi and other soup cubes; (g) Peas; (/;) Methi, arvi leaves

List B*—Foods to be consumed in moderate amounts*

(a) Beans, beet root, cauliflower, bathua, cabbage, carrot, onion, potatoes, radish, sweet potatoes, lowki, brinjal, cucumber, bhindi, pumpkin, tomatoes; *(b)* banana, grapes, guava, mango, papaya, apple *List* C*—Unrestricted foods*

(a) Arrowroot, cornflour, sago, Custard powder; *(b)* Sugars, honey, Jam, marmalade, jellies; (c) Butter, cooking fat and oil; *(d)* Tea, coffee, squash; (e) Salt, pepper, vinegar, spices, curry powder*;(f)* Lowki, tori, tinda

APPENDIX B— *Low Methionine Diet for a 10 kg Child (Methionine allowance 25-45 mg/kg. Cal: 100-120/kg; Protein 2g/kg)*

	Cal	Protein (g)	Methionine (mg)
Breakfast			
Buffalo milk 100 ml	115	4.0	100
+ 1tsf sugar	20	-	-
Sago khichari (10 g sago, 25 g Potato, 1 tsf oil	110	1.0	6.0
Lunch			
Rice 50 g cooked & drained + 1 tsf ghee	220	3.5	25
Vegetable 1 Katori + 1tsf ghee	50	-	10
Curd 50 ml +	60	2.0	50
Fruit 1	50	-	-
Tea			
50 ml milk	60	2.0	50
+ 1tsf sugar	20	-	-
2 Arrowroot biscuits	50	-	-
Dinner			
Rice 50 g cooked & drained + 1 tsf ghee	220	3.5	25
1 Katori vegetable	50	1.0	10
1 Katori dal Masur cooked (25 g raw)	70	1.25	10
Sweet Semolina kheer (20 g semolina + 25 ml milk + 1 tsf sugar)	120	3.50	55
Total	1215	21.75	341