Erythrocyte Formiminotransferase Activity in Formiminotransferase Deficiency Syndrome

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Formiminotransferase activity was estimated on erythrocyte lysates obtained from two cases with the formiminotransferase deficiency syndrome, and was found to be markedly decreased in both the cases as compared with that of controls. This finding suggested that an estimation of formiminotransferase activity of erythrocyte lysates might be used as a tool for diagnosis of this syndrome.

Clinical and biochemical findings of the third case of formiminotransferase deficiency syndrome were described.

Formiminotransferase deficiency syndrome^{1,2} is diagnosed on the basis of the three criteria consisting of 1) mental retardation, 2) hyperfolic-acidemia and 3) formiminoglutamic aciduria with or without an oral histidine loading.

However, the diagnosis of this syndrome is confirmed when a decreased activity of formiminotransferase is actually demonstrated in liver specimens biopsied from the patients in question.^{1,2}

The present study revealed a decrease in the formiminotransferase activity of erythrocytes from two cases with formiminotransferase deficiency syndrome and suggested the possibility that an estimation of the formiminotransferase activity of erythrocytes might be a useful tool for a diagnosis of this syndrome.

METHODS AND MATERIALS

Preparation of erythrocyte lysates

The hemolysate was prepared according to Bertino *et al.*'s method.³ A few ml of blood were taken into a heparinized syringe and erythrocytes were separated by centrifugation, then washed twice with 0.15 M KCl solution. To one volume of the washed erythrocytes were added two volumes of 0.001 M potassium phosphate buffer of pH 8.0. The content was mixed well and then centrifuged. The supernatant fraction was adjusted to be 10.5 g per 100 ml in a hemoglobin concentration by the addition of 0.001 M potassium phosphate buffer of pH 8.0 – the hemolysates.

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Assay system for formiminotransferase activity of the hemolysates

Formiminotransferase activity of the lysates was estimated according to Tabor and Wyngarden's procedures.⁴

Composition of the assay system was as follows; 1.0 ml of formiminoglutamic acid solution (2.0 μ moles of formiminoglutamic acid were dissolved in one ml of 0.1 N HCl), 0.2 ml of DL-tetrahydrofolic acid solution (88 mg of DL-tetrahydrofolic acid were dissolved in a mixture of 18 ml of mercaptoethanol and 2 ml of 0.2 N KOH), 0.5 ml of 0.1 M potassium phosphate buffer of pH 7.2, and 0.10 ml, 0.15 ml and 0.20 ml of the hemolysates. For the reference tubes, 1.0 ml of 0.1 N HCl was used in place of 1.0 ml of the formiminoglutamic acid solution.

The reaction mixture was kept in a water bath at 25°C for 30 minutes, then the reaction was stopped by the addition of 0.5 ml of 10% trichloroacetic acid. Then the content was kept in a boiling water bath for 55 seconds, then put into an iced water for 5 minutes. After centrifugation the supernatant was used for assay of N^{5,10}-methenyltetrahydrofolic acid by using an automatic recording spectrophotometer. The formiminotransferase activity was expressed as an increment of the optical density due to N^{5,10}-methenyltetrahydrofolic acid.

TABLE 1. *Formiminotransferase activity of erythrocyte hemolysates in formiminotransferase deficiency syndrome*

Group	No. of case	Name, age and sex	Increments of optical density due to N ^{5,10} -methenyltetrahydrofolic acid		
			0.1*	0.15*	0.20*
Formimino-transferase deficiency syndrome	1	C. K. 3 y. 8 m. ♀	0.02	0.055	0.11
	2	U. J. 9 m. ♀	0.02	0.07	0.115
Controls	3	H. S. 8 m. ♀	0.135		0.315
	4	S. S. 9 m. ♂	0.09	0.135	0.21
	5	K. H. 1 y. 2 m. ♂	0.09	0.15	0.30
	6	M. M. 1 y. 5 m. ♀	0.08	0.12	0.18
	7	T. T. 1 y. 5 m. ♂	0.09	0.19	
	8	S. K. 1 y. 6 m. ♂	0.11	0.27	0.36
	9	O. K. 1 y. 7 m. ♀	0.09	0.16	0.32
	10	K. K. 2 y. 0 m. ♀	0.10	0.20	0.27
	11	K. Y. 4 y. ♂	0.115	0.26	0.38
	12	T. Y. 5 y. ♂	0.13	0.25	0.33
	13	Y. Y. 5 y. ♀	0.24	0.34	0.375
	14	S. T. 6 y. ♂	0.10	0.19	0.32
	15	S. K. 7 y. ♂	0.09	0.20	0.36

* Amounts of erythrocyte lysate used for the assay (ml)

All steps of the assay procedures up to the incubation step were carried out at 4°C and accomplished within 30 minutes after blood specimens were taken.

Materials

The erythrocyte lysates were prepared from two cases with formiminotransferase deficiency syndrome (Cases 1 and 2) and from 13 controls (Cases 3–15) (cf. Table 1).

Case 1, C.K., a 3-year-8-month-old girl was the same patient as had been reported¹ as the first case of formiminotransferase deficiency syndrome in 1963. A marked decrease in the formiminotransferase activity was demonstrated in the liver tissues biopsied from that patient when 8 months old. Since then she has been admitted in our hospital and is living with a severe mental retardation.

The second case of formiminotransferase deficiency syndrome,² a 1-year-7-month-old girl, had died, so is not available for the present investigation.

Case 2, U.J., listed in Table 1, was a 9-month-old girl who was the third case of formiminotransferase deficiency syndrome experienced in our clinic up to the present time. Clinical and biochemical findings of this patient were given in the appendix.

Cases 3–15 in Table 1 were control subjects who showed no abnormality in folic acid metabolism.

RESULTS AND DISCUSSION

As will be seen in Table 1 and Fig. 1, it was clearly demonstrated that the erythrocyte formiminotransferase activity was markedly decreased in two cases of formiminotransferase deficiency syndrome (Cases 1 and 2) as compared with that in controls (Cases 3–15).

A diagnosis of formiminotransferase deficiency syndrome was established in Cases 1 and 2, which fulfilled such three criteria for the diagnosis as 1) mental retardation, 2) hyperfolic-acidemia and 3) formiminoglutamic aciduria following an oral dose of histidine.^{1,2} In addition, a marked decrease in formiminotransferase activity had been demonstrated¹ in liver tissues biopsied from Case 1.

Within our literature survey there has been no report dealing with formiminotransferase activity of human erythrocytes. A probable existence of formiminotransferase in human erythrocytes is, however, suspected in view of Bertino *et al.*'s report³ where the existence of some enzymes related to folate metabolism such as formate activating enzyme, serine hydroxymethylase and N⁵,¹⁰-methenyltetrahydrofolic acid-dehydrogenase in human erythrocytes was described.

Fig. 1. Formiminotransferase activity of erythrocyte lysates.
 ●—● Cases with formiminotransferase deficiency syndrome
 x—x Controls

APPENDIX

The third case of formiminotransferase deficiency syndrome

U.J., a 9-month-old girl, listed as Case 2 in Table 1 of this paper, was the third case of formiminotransferase deficiency syndrome experienced in our clinic up to the present time.

The patient (U.J.) was born on Nov. 13, 1964 at full term, weighing 2,900 g, after an uneventful pregnancy and delivery. She was an only child of healthy and nonconsanguineous parents. The patient was unable to recognize her mother but sat alone when 3 months old, and the mother wondered about a defective vision of her child.

On admission, June 17, 1965, when 7 months old, physical examination revealed a well nourished girl. Bodyweight was 7.5 kg, height 65 cm and

circumference of head 42 cm. All these figures were within normal range. A horizontal nystagmus was noticed bilaterally. Auscultation of the chest revealed neither cardiac murmurs nor rales. Abdomen was slightly distended. Liver and spleen were not palpable. Extremities were not rigid and no pathological reflexes were elicited.

Fundoscopic examination revealed retinal degeneration of unknown origin.

Electroencephalograms (sleeping records) revealed 2-3 cps H.V.S. and 25 cps fast waves in all areas, sporadic spikes and sporadic sharp waves in bilateral fronto-parieto-temporal areas, and 14 cps positive spikes in both parietal areas. These findings suggested a diffuse brain damage.

Chromatographic analysis of urinary amino acids revealed no abnormality except a slight increase in β -amino-isobutyric acid. Urinary tests for sugar and protein were negative, and the FeCl_3 and Millon reactions were negative.

TABLE 2. *Blood pictures of Case 2 (U.J.)*

Date	17/VI, 1965	30/VIII, 1965
Red cell count ($\times 10^4$ per mm^3)	478	478
Hemoglobin (g per 100 ml)	13.6	14.5
Hematocrit (%)		47
White cell count (per mm^3)	11,850	17,750
Basophils (%)	0	0.5
Eosinophils (%)	2	1.0
Lymphocytes (%)	67	66.5
Monocytes (%)	6	2.5
Neutrophils (%)	25	29.5
Nuclear pattern of neutrophils (%)	I	14
	II	46
	III	24
	IV	16
	V	0

TABLE 3. *Chemical composition of serum of Case 2 (U.J.)*

Total protein (g per 100 ml)	7.4
Albumin (%)	74.3
α_1 -Globulin (%)	2.4
α_2 -Globulin (%)	11.2
β -Globulin (%)	4.8
γ -Globulin (%)	7.3
Sodium (mEq/l)	145
Potassium (mEq/l)	4.5
Chloride (mEq/l)	113.8
Urea nitrogen (mg per 100 ml)	9.7
Total lipids (mg per 100 ml)	757
{ Phospholipids (mg per 100 ml)	257
{ Cholesterol (mg per 100 ml)	230
Vitamin B ₁₂ (m γ /ml)	0.47

Roentgenograms of the chest, bones and skull revealed no abnormality. Peripheral blood pictures, shown in Table 2, revealed no abnormality. Quantitative estimation of serum protein, sodium, potassium, chloride, urea, lipids and vitamin B₁₂ revealed no abnormality (cf. Table 3). Serum folic acid activity, assayed by using *L. casei*, was found to be 85 mγ/ml. Urinary FIGLU following an oral dose of L-histidine-monohydrochloride (0.33

TABLE 4. *Urinary FIGLU following an oral dose of L-histidine-monohydrochloride 0.33 g per kg of bodyweight in Case 2 (U.J.)*

	FIGLU (μmoles)	
	per 8 hour urine	per ml of urine
7/VIII 1965	113.5	2.27
27/VIII 1965	34.2	1.68
3/IX 1965	180.0	1.50
20/X 1965	192.4	3.12

TABLE 5. *Serum folates and urinary FIGLU following an oral dose (20 g) of L-histidine-monohydrochloride in the parents of Case 2 (U.J.)*

	FIGLU (μmoles)		Serum folate (<i>L. casei</i>) (mγ/ml)
	per 8 hour urine	per ml of urine	
Father	110.96	0.19	10.0
Mother	44	0.08	15.9

TABLE 6. *Blood pictures of the parents of Case 2 (U.J.)*

	Father	Mother
Red cell count (× 10 ⁴ per mm ³)	505	458
Hemoglobin (g per 100 ml)	16.3	15.9
White cell count (per mm ³)	15,600	15,900
Eosinophils (%)	2	1
Lymphocytes (%)	35	41
Monocytes (%)	8	5
Neutrophils (%)	55	53
Nuclear pattern of neutrophils (%)	I	14
	II	17
	III	42
	IV	32
	V	14
	2	9
		0

Fig. 2. The pedigree of the "U.J." family.

●: The patient

⊗: Cases with 'night blindness'

g/kg of bodyweight) was found to be abnormally increased (cf. Table 4).

Family study: Urinary FIGLU following an oral dose (20 g) of L-histidine-monohydrochloride was estimated on both the parents of Case 2 (U.J.) and was found to be slightly increased in only the father (cf. Table 5). Serum folate activity (L. casei) of the parents was found to be normal (cf. Table 5). Peripheral blood pictures of the parents also showed no abnormality (cf. Table 6).

The parents were not consanguineous but consanguinity was found among ancestors of both maternal and paternal sides (cf. Fig. 2). Two sisters of maternal grandfather were said to be suffered from 'night blindness', but details of the illness were not clear.

References

- 1) Arakawa, Ts., Ohara, K., Kudo, Z., Tada, K., Hayashi, T. & Mizuno, T. "Hyperfoliacidemia with formiminoglutamic-aciduria following histidine loading" suggested for a case of congenital deficiency in formiminotransferase. *Tohoku J. exp. Med.*, 1963, **80**, 370-382.
- 2) Arakawa, Ts., Ohara, K., Takahashi, Y., Ogasawara, J., Hayashi, T., Chiba, R., Wada, Y., Tada, K., Mizuno, T., Okamura, T. & Yoshida, T. Formiminotransferase-deficiency syndrome: A new inborn error of folic acid metabolism. *Ann. paediat.*, 1965, **205**, 1-11.

- 3) Bertino, J.R., Simmons, B. & Donohue D.M. Purification and properties of the formate-activating enzyme from erythrocytes. *J. biol. Chem.*, 1962, **237**, 1314–1318.
 - 4) Tabor, H. & Wyngarden, L. The enzymatic formation of formiminotetrahydrofolic acid, 5,10-methenyltetrahydrofolic acid, and 10-formyltetrahydrofolic acid in the metabolism of formiminoglutamic acid. *J. biol. Chem.*, 1959, **234**, 1830–1846.
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