FOLIC ACID, VITAMIN B_{12} AND VITAMIN B_{12} BINDING PROTEINS IN PATIENTS WITH NEUROBLASTOMA

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INTRODUCTION

Neuroblastoma is a common malignant tumor of children next to leukemia, lymphoma and brain tumors which accounts for about 10% of childhood cancer in Thailand (Hathirat *et al.*, 1985). This tumor constitutes a rare example of malignant undifferentiated tissue which can mature to a more differentiated form to ganglioneuroma or can regress spontaneously. The formal treatment consists of surgical removal of the tumor when possible. Radiation therapy and chemotherapy are also given as specific and palliative measures.

In 1959 Bodian reported a 50% response rate following high daily dose of vitamin B_{12} in 15 of 28 patients with neuroblastoma (Bodian, 1959). This author again reported benefit with vitamin B_{12} in 40 of 73 children with neuroblastoma so treated (Bodian, 1963). However, subsequent study has not been able to confirm this report (Sawitsky and Desposito, 1965). As data on vitamin B_{12} status in patients with neuroblastoma is not available at present, the effect of vitamin B_{12} treatment therefore could not be assessed. The objective of the present study was to determine vitamin B_{12}, vitamin B_{12} binding proteins, serum and red cell folate levels in patients with neuroblastoma.

MATERIALS AND METHODS

The studies were performed on 18 patients with neuroblastoma of both sexes. They were randomly selected at the outpatient clinic, Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Bangkok. Their ages ranged from 8 months to 14 years. There were 11 males and 7 females.

Venous blood samples were taken from the antecubital vein for determination of serum vitamin B_{12}, vitamin B_{12} binding proteins (transcobalmins TC), serum and red cell folate levels, white blood cells and red blood cells. Bone marrow puncture and liver scan were also determined in these patients.

Serum vitamin B_{12} levels were determined by the radiositope dilution and coated charcoal technique (Lau *et al.*, 1965). Transcobalamins were fractionated and quantitatively measured by the method described by Selhub *et al.*, (1976). The unsaturated vitamin B_{12} binding capacity (UBBC) was calculated from the sum of TCI, TCII and TCIII. Total vitamin B_{12} binding capacity (TBBC) was calculated from the sum of serum vitamin B_{12} and UBBC.

Red cell and serum folate levels were determined by *Lactobacillus casei* method of Hoffbrand *et al.*, (1966) and Waters and Mollin (1961), respectively.

RESULTS

The results of serum vitamin B_{12}, serum and red cell folate levels in 18 patients with neuroblastoma are shown in Table 1. The mean serum vitamin B_{12} value (564 ± 399 pg/ml) in these patients was slightly but not signi-

Table 1

Serum vitamin B_{12}, serum and red cell folate levels in patients with neuroblastoma.

No.	Age (years)	Ht (%)	Hb (g%)	WBC ($c.mm^{-1}$)	Vit. B_{12} (pg/ml)	Folate (ng/ml)	
						Serum	RBC
1.	14	40	10.9	10300	781	3.5	169
2.	2.8	31	9.3	6900	859	3.8	141
3.	2	15	5.0	4400	256	6.2	653
4.	4	37	5.4	7500	283	11.3	240
5.	1.5	25	8.3	10400	158	15.6	417
6.	4	29	9.6	24300	164	4.7	358
7.	8	23	7.6	7000	846	0.3	378
8.	4	31	10.0	7500	421	4.0	1013
9.	6	18	7.9	4800	949	6.4	711
10.	6	36	12.0	7500	117	6.1	106
11.	8.5	33	9.8	13000	193	5.8	563
12.	4	27	9.3	7800	151	2.9	571
13.	4	30	10.0	15000	452	6.5	864
14.	5	20	6.2	8700	290	5.5	563
15.	1	36	12.0	9050	657	11.2	384
16.	3	28	9.5	8000	1340	11.3	340
17.	12	25	8.3	7400	1343	15.3	741
18.	2	15	4.9	9100	829	3.3	294
Mean	—	28	8.7	—	564	6.9	473
± S.D.		7	2.2		399	4.3	255

ficantly lower than that (629 ± 160 pg/ml) of normal subjects ($p > 0.05$). As all serum vitamin B_{12} in these patients were above 150 pg/ml, therefore none of them was considered as vitamin B_{12} deficient. There was no relationship between serum vitamin B_{12} levels and white blood cells, hemoglobin or hematocrit ($r = -0.019$, -0.004 and -0.148, respectively). Both mean values of serum and red cell folate levels were significantly lower ($p < 0.05$) than the mean values 9.8 ± 5.0 and 684 ± 235 ng/ml, respectively, in normal human subjects. Only 2 out of 18 patients had serum folate levels lower than 3 ng/ml and none of them had red cell folate levels less than 100 ng/ml.

Table 2 shows the mean values of serum UBBC, TBBC and TC levels in 8 patients with neuroblastoma. Serum UBBC levels in these patients were slightly but not significantly higher than that of the normal ($p > 0.05$) while serum TBBC levels were nearly the same in both groups. Only TCI showed a significantly higher value while TCII and TCIII were not significantly lower than those of normal subjects.

DISCUSSION

The results in the present study showed that neuroblastoma patients had slightly but not significantly lower serum vitamin B_{12} levels

Table 2

Mean value ± S.D. of serum UBBC, TBBC and TC levels in 8 patients with neuroblastoma and in 60 normal subjects.

	Normal	Neuroblastoma	P. value
UBBC (pg/ml)	1589 ± 389	1720 ± 720	N.S*
TBBC (pg/ml)	2218 ± 458	2288 ± 725	
Transcobalamin (%)			
TCI	13 ± 3	22 ± 4	< 0.01
TCII	78 ± 5	69 ± 7	< 0.01
TCIII	9 ± 3	9 ± 4	N.S*
Transcobalamin (pg/ml)			
TCI	205 ± 61	377 ± 177	< 0.001
TCII	1231 ± 318	1215 ± 583	N.S*
TCIII	153 ± 77	133 ± 60	

*N.S. = not significant.

but none of them had serum vitamin B_{12} less than 150 pg/ml. These findings indicated that their serum vitamin B_{12} levels were within the normal limits and there was no evidence of vitamin B_{12} deficiency in this group of patients.

The finding of a significantly increased TCI and slightly but not significantly elevated UBBC levels indicated a compensatory mechanism. In order to raise the low serum vitamin B_{12} to the normal level, the TCI has to be increased for binding more endogenous vitamin B_{12} in the serum. Therefore, the sum of serum vitamin B_{12} and TCI (assuming all endogenous vitamin B_{12} is bound to TCI) in these patients (941 pg/ml) was slightly higher than that (834 pg/ml) of normal subjects. This compensatory mechanisms have been reported to occur in various conditions described earlier (Areekul *et al.*, 1977).

As mentioned earlier, a good response has been reported in patients with neuroblastoma following a high daily dose of vitamin B_{12} treatment (Bodian, 1959; 1963). The rationale for using vitamin B_{12} was that since vitamin B_{12} was an essential factor for the normal maturation of hematopoietic cells, it might make neuroblastoma mature to ganglioneuroma. However, the reasoning proved wrong and the mechanism of good response of the tumor to regress is unknown. Later study showed no increase in the remission rate either when vitamin B_{12} was used alone or in conjunction with X-ray or other chemotherapeutic agents in patients with advanced neuroblastoma (Sawitsky and Desposito, 1965). Although the malignant neuroblast cells show very aggressive malignancy, they sometimes become differentiated into mature ganglion cells so that a benign ganglioneuroma is produced. Spontaneous remission of widely disseminated neuroblastoma has been reported even after it has metastasized. It was therefore highly possible that the good response after giving high dose of vitamin B_{12} was due to the fact that the majority of the patients treated were infants who do well irrespectively of treatments. Since there is no evidence of vitamin B_{12} deficiency in patients with neuroblastoma, treatment with vitamin B_{12} would have no direct beneficial effect in these patients.

The present study showed that the mean serum folate level in patients with neuroblastoma was significantly lower than that of the normal subjects. These findings are in accordance with a report of low serum folate level in patients with malignancy (Rao *et al.*, 1963). Serum folate concentration has also been found to be low in 122 of 306 patients with malignant disease (Kershaw and Girdwood, 1964). The low serum folate concentration was possibly due to the increased urinary excretion of formiminoglutamic acid as has been reported to occur in 88 out of 168 patients with malignancy (Cary *et al.*, 1964; Dymock, 1964). Studies on the mean hepatic folate level in patients with normal serum folate also showed a higher value than that of patients with carcinoma (Romaine, 1960; Leevy *et al.*, 1965; Chanarin *et al.*, 1966). In the present study only 2 out of 18 patients with neuroblastoma had low serum folate values and none of them had red cell folate levels lower than the lowest limit of normal subjects. This indicates that these patients were only in the state of negative folate balance but not folate deficiency. A progressive decline of red cell folate level could occur only in widespread cancer of long standing.

SUMMARY

Serum vitamin B_{12}, serum and red cell folate and serum vitamin B_{12} binding proteins were determined in 18 patients with neuroblastoma, with ages ranging from 8 months to 14 years. A mean value of serum vitamin B_{12} level was slightly but not significantly lower than that of the normal subjects but all of them had serum vitamin B_{12} levels over 150 pg/ml. There was no relationship between serum vitamin B_{12} levels and hemoglobin, hematocrit or white cells. Transcobalamin I (TCI) was significantly increased resulting in slightly elevated UBBC and normal TBBC levels in these patients. This could be a compensatory mechanism for the low serum vitamin B_{12} by increasing the unsaturated vitamin B_{12} binding capacity of TCI. All these findings indicated that the status of vitamin B_{12} in patients with neuroblastoma was within the normal limits. Treatment of neuroblastoma by giving a high dose of vitamin B_{12} would therefore not give any direct therapeutic effect. Both serum and red cell folate concentrations were significantly lower in the group of patients. As only 2 out of 18 patients had low serum folate and none of them had red cell folate lower than the lower limit of normal subjects; therefore these patients were only in the state of negative folate balance.

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