Original Article

Effect of nutrition support on immunity in paediatric patients with beta-thalassaemia major

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Nutritional deficiencies have been variably observed in thalassaemia and the aetiology of many of the immune abnormalities in thalassaemic children are poorly defined. Therefore, we tested the hypothesis that certain immune abnormalities have a nutritional basis. Nutritional status, selective quantitative and functional indices of immunity were studied in twelve children (7 females, 5 males; mean age 28 months, SD 5 and range 19.8-35.5), with thalassaemia major before and after a one month period of intensive nutrition support (the study diet consisted of 'Enfapro' liquid formula (Mead Johnson) with added dextrose and corn oil to achieve a caloric density of 1.1 kcal/cc in addition to vitamins and minerals). Each child was provided approximately 150 kcal/day and 4 g of protein/day. Lymphocyte proliferation to Concanavalin A (Con A) (P = 0.008) and Purified Protein Derivative (PPD) (P = 0.002) was depressed upon entry into the study, however the response to Con A attained normal values by the end of the intervention. Compared to baselines, the proliferative response to Con A (P=0.005) and Phytohemagglutinin A (PHA) (P = 0.031) both improved after the nutrition support. Although there was no general correlation of zinc status with lymphocyte proliferation, normal baseline zinc status was associated with improvement of proliferation. The %CD4 increased (P = 0.036), primarily because of a decrease in total lymphocytes and to lesser extent a decrease in CD8 lymphocytes. Serum immunoglobulin concentrations were found to be elevated on admission but were not significantly affected by the nutrition intervention. C3 concentrations were uniformly depressed on admission but increased by the end of the study protocol (P = 0.037). C4 and CH50 activity were not significantly influenced by the intervention. In conclusion, children with beta thalassaemia have abnormalities of lymphocyte function as well as key complement components that are responsive to nutrition support. In addition, zinc status appears to have an important role in lymphocyte function in these children.

Key Words: thalassaemia, malnutrition, nutrition, immunity, children, Chiang Mai, Thailand

Introduction

Death due to infections is common in children with beta thalassaemia major.[1,2] It has been shown that serum immunoglobulin levels of children with beta thalassaemia may be normal or increased.[3-6] There are some reports that complement level, cell mediated immunity and neutrophil function in these patients are impaired or normal. Children with beta thalassaemia major have growth failure, delayed puberty for poorly defined reasons which are probably related to undernutrition.[7,8] It has been reported that there are multiple deficiencies of vitamins such as vitamin A, E, folic acid, B12 and also zinc.[9-15] Interestingly, nutritional status is rarely cited as having a possible role in the immune deficits in these children. Despite the coexistence of beta thalassaemia with nutrient deficiencies, malnutrition as an important cause of immune deficits has not been adequately studied. We therefore designed a study to investigate the effect of nutrition support on specific immune indices in patients with beta thalassaemia major.

Methods

The study subjects included 5 boys and 7 girls with homozygous beta thalassaemia. They were randomly selected from the thalassaemia clinic at Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand. All children were non-splenectomized, aged 1 to 3 years and were also HIV seronegative. Informed consent was obtained from the parent. The study protocol was approved by the Human Ethics Committee from Chiang Mai University, Thailand. All of the study parameters were assessed at baseline and after a one month period of intensive nutritional intervention.

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Anthropometry

Weight (WT), height (HT), mid arm circumferenc (MAC) and skinfold thicknesses [triceps (TSF), subscapular (SSF)] were measured and compared to National Center for Health Statistics (NCHS) references.[16] Percentage W/H ([subject's weight divided by reference weight at 50th percentile] X 100) and percentage H/A ([subject's height divided by reference height at 50th percentile] X 100) were calculated using Waterlow's classification.[17] Body mass index (BMI), arm muscle area (AMA), arm fat area (AFA), lean body mass (LBMSF) and fat mass (FMSF) were calculated from WT, HT, MAC, TSF, SSF measurements. Standard deviation scores or Z scores of W/H, H/A and W/A were also calculated.

Immunology

Percent and absolute number of CD4 and CD8 were measured. T lymphocyte functions were studied using the stimulation of T cells by Con A, PHA, PPD and allogenic cells. Serum immunoglobulins: IgG, IgA, IgM and complements C3, C4, CH50 were also measured.[18] All indices were compared to reference values.[18-21] Flow cytometry was used to determine the percentage and absolute number of T cells expressing the antigens CD4 (helper/inducer cell marker) and CD8 (suppressor/cytotoxic cell marker) using suitable monoclonal antibodies. PPD was used as a recall antigen as all subjects and controls received BCG immunization at birth. All data are presented as D-cpm (background cpm in unstimulated cultures subtracted from cpm in stimulated cultures). Serum IgG, IgA, IgM, C3, and C4 concentrations were determined by kinetic turbimetric measurement (Turbiquant, Behring) and total haemolytic complement activity (CH_{50}) was measured using a sensitized sheep red blood cell lysis assay.

Plasma zinc

Plasma zinc concentration was determined by atomic absorption spectrophotometry (Perkin-Elmer Model 3100).

Nutritional intervention

The subjects were admitted for one month to the metabolic ward for nutrition support. They were fed with Enfapro liquid formula (from Mead Johnson company) with added dextrose and corn oil in order to achieve a caloric density of 1.1 kcal/cc in addition to vitamins and minerals. Each child had one-to-one nursing. They were encouraged to consume the study diet with 150 kcal/kg/day and 4 g of protein/kg/day. Syrup zinc sulphate was given with the dosage as elemental zinc 1.5 mg/kg/day in order to meet 3 times of the Recommended Dietary Allowance.

Statistical analysis

Fisher's exact test was used to examine differences in proportions. Mann-Whitney U test was used for group comparisions of continuous data. Wilcoxon ranked sums test was used to compare the measurement data before and after nutritional intervention. Regression analysis was used to investigate relationships between dependent and independent variables. The statistical significance was at $P < 0.05$.

Results

The mean age of the study subjects was 28 months on admission (Table 1). Only height and percentage height for age, were not significantly increased after nutrition intervention. Weight, weight for age and weight for height were significantly improved after nutrition intervention. On admission, five children were mildly malnourished, which was corrected after nutrition intervention. After nutrition intervention, there was about a 10% increase in lean body mass and about a 30% increase in fat mass (Table 2).

Table 1. Characteristics of thalassaemia children before and after nutrition intervention

	Baseline		After intervention		
Characteristic	Mean	SD	Mean	SD	*P*
Age (m)	27.9	5.0	28.8	5.0	-
Weight (kg)	10.0	1.7	12.1	1.7	0.003
Height (cm)	84.7	6.0	85.1	6.0	0.213
Height for age (Z score)	-1.12	1.0	-1.14	1.1	0.845
Weight for age (Z score)	-1.48	1.0	-0.70	0. 9	0.003
Weight for height (Z score)	-0.94	0.8	0.05	0.7	0.003

Table 2. Changes of body composition after nutrition intervention

	Percentage increased	Md	SD	*P*
WT (kg)	11	1.20	0.18	0.003
BMI (kg/m^2)	10	1.57	0.56	0.003
MAC (cm)	8	1.14	0.32	0.003
AMA (cm^2)	9	0.96	0.67	0.004
LBMSF (kg)	7	0.66	0.20	0.003
TSF (mm)	27	2.02	0.90	0.004
SSF (mm)	27	1.60	0.90	0.004
AFA (cm^2)	35	1.66	0.54	0.003
FMSF (kg)	32	0.54	0.15	0.003

Md: mean difference, SD: standard deviation, WT: weight, BMI: body mass index, MAC: mid-arm circumference, AMA: arm muscle circumference, LBMSF: lean body mass calculated from skinfold thickness, TSF: triceps skinfold thickness, SSF: subscapular skinfold thickness, AFA: arm fat area, FMSF: fat mass calculated from skinfold thickness.

Serum immunoglobulins were higher than normal values on admission and were decreased after nutrition intervention (Table 3). For complement, C3, C4, CH50 were low but only C3 was significantly improved after nutrition intervention. For the study of lymphocyte, lymphocyte count and % lymphocyte were not changed after nutrition intervention (Table 4). However, absolute lymphocyte was decreased after nutrition intervention. For CD4 and CD8, the absolute CD4 and CD8 were not changed after nutrition intervention.

Table 3. Measures of humoral immunity in thalassemic children before and after nutrition intervention

Measure	Reference Standard	Before Mean ± SD	After Mean ±SD	*P**
IgG (mg/dl)	892 ± 183	1405 ± 1332	962 ± 396	0.255
IgM (mg/dl)	61 ± 19	173 ± 112	139 ± 113	0.154
IgA (mg/dl)	71 ± 37	103 ± 54	93 ± 41	0.625
C4 (mg/ml)	200 - 500	248 ± 103	208 ± 100	0.126
C3 (mg/ml)	550 - 1200	456 ± 63	498 ± 69	0.037
CH_{50} (U/ml)	20 - 40	29 ± 6	29 ± 6	1.000

Reference values for immunoglobulins expressed as mean ± SD, complement reference values expressed as normal range. *Differences between Before and After nutrition intervention.

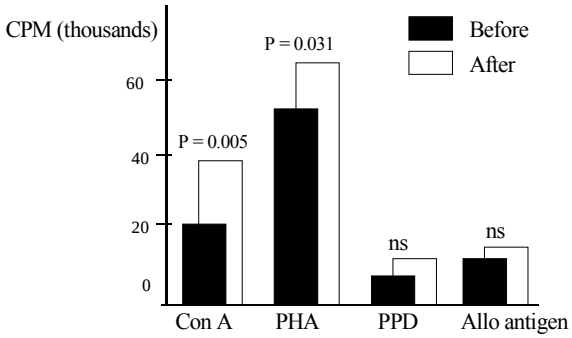

Figure 1. Lymphocyte responsiveness (mean ± SEM, n = 12) before and after nutrition intervention

Only the %CD4 was significantly increased, but not the %CD8. This is because the absolute lymphocyte was significantly reduced. However, the CD4 to CD8 ratio was not changed. For the study of lymphocyte blastogenesis (Table 5), after nutrition intervention mitogen ConA and the antigen PPD were significantly increased, but not the PHA and alloantigen. However, all of the values were increased after nutrition intervention. For the lymphocyte responsiveness before and after nutrition intervention (Fig.1), only ConA and PHA groups were significantly increased, but not the PPD and alloantigen groups.

We divided our subjects into two groups: a group with normal plasma zinc level and a group with low plasma zinc level. In the group with the normal plasma zinc level, ConA, PHA and PPD groups were significantly increased, but not the alloantigen group (Fig. 2). In the group with the low plasma zinc levels, none of the subjects were significantly improved (Fig. 3).

Discussion

The results of this study demonstrate that children with homozygous beta thalassaemia have cell-mediated and humoral immunologic abnormalities which in part have a nutritional basis. The relatively impaired lymphocyte response to the mitogens Con A and PHA in the thalassemic children compared

Table 4. Lymphocyte analysis in thalassemic children before and after nutrition intervention

Measurement	Reference Standard	Before	After	*P**
Leukocyte count ($x10^3$ cells/mm^3)	7.8 (7-10)	11.2 (9-14)	10.0 (8-12)	0.099
% Lymphocytes	46% (38-53)	36% (31-46)	35% (30-44)	0.575
% CD4	37% (30-40)	32% (25-35)	37% (30-40)	0.036
% CD8	29% (25-32)	27% (25-30)	26% (22-36)	0.500
Absolute L ($x10^3$ cells/mm^3)	3.6 (3-5)	3.7 (3-6)	3.2 (3-4)	0.042
Absolute CD4 ($x10^3$ cells/mm^3)	1.6 (1-1.8)	1.2 (1.0-1.6)	1.2 (0.9-1.3)	0.263
Absolute CD8 ($x10^3$ cells/mm^3)	0.9 (0.8-1.5)	1.0 (0.8-1.8)	0.8 (0.7-1.1)	0.484
CD4/CD8	1.3 (1-1.6)	1.2 (0.8-1.5)	1.4 (1-1.8)	0.411

Data presented as median with ranges from 25th to 75th percentiles in parentheses. *Difference between Before and After nutrition intervention.

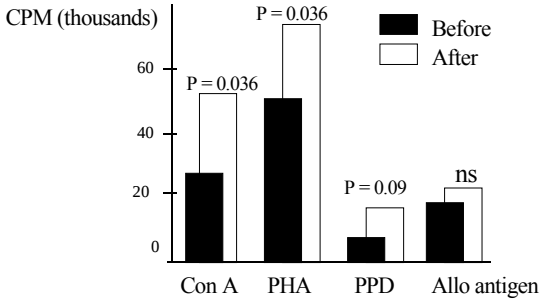

Figure 2. Lymphocyte responsiveness (mean ± SEM, n = 6) before and after nutrition intervention in children with normal zinc (>60 μg/dL) on admission

to controls, together with the improvement in transformation after the nutritional intervention, demonstrates a depressed cell-mediated immunity of nutritional origin. Response to the antigen, PPD, did not significantly improve over the study period. This might be due to incomplete correction of all nutritional deficiencies present and/or other concomitant factors affecting antigen presentation and recognition at the time of immunization with BCG.

Zinc appears to be one of the important determinants, with baseline plasma zinc predicting a response to the nutrition support. Unlike the children with normal serum zinc levels on admission, the children with low serum zinc exhibited no improvement in lymphocyte blastogenic response to Con A and PHA after the nutritional intervention, even though serum zinc levels increased to within the normal range. One possible explanation is that a normal plasma zinc concentration might not accurately reflect lymphocyte zinc sufficiency, and a longer period of supplementation might be required to restore lymphocyte zinc.[22] Unfortunately, lymphocyte zinc was not measured in this study. Further study is necessary to define the

Table 5. Lymphocyte blastogenesis in thalassaemic children before and after nutritional intervention compared to control values

Stimulant	Control n = 12 Mean SEM	Before n = 12 Mean SEM	After n = 12 Mean SEM	*P**
Con A	33,123 ± 3196	20,950 ± 2409†	38,625 ± 5657	0.008
PHA	56,706 ± 3854	53,173 ± 3743	64,498 ± 4881	0.164
PPD	22,254 ± 892†	6806 ± 1148	10,748 ± 2991	0.002
Allo-antigen	12,364 ± 1563	10,235 ± 2058	12,487 ± 3533	0.683

Data presented as mean ± SEM. *Comparison of groups by Kruskal-wallis one-way ANOVA. †Values with superscript significantly differ from other two groups.

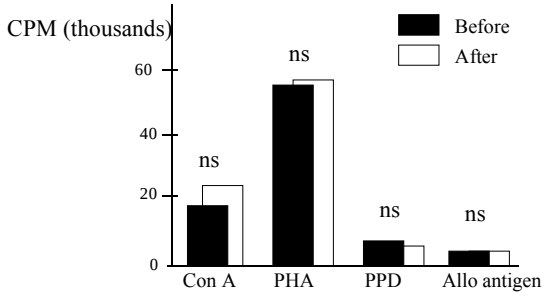

Figure 3. Lymphocyte responsiveness (mean ± SE, n = 6) before and after nutrition intervention in children with low zinc (<60 μg/dL) on admission

time required for restoration of lymphocyte zinc and to explore the relationship with lymphocyte function. The modest increases in immunoglobulins in our children on admission are similar to that reported in other studies, and has been speculated to be the result of polyclonal B cell stimulation in response to multiple blood transfusions.[23,24] While the difference after the nutritional intervention in immunoglobulin levels was not significant in our children, there was a definite trend towards the normal range, similar to the change in immunoglobulin concentrations that occurs in children with primary protein energy malnutrition after nutritional rehabilitation.

The decreased C4 and C3 levels were not profound enough to significantly affect the haemolytic activity of the classical pathway. The decreased C3 concentrations might reflect either decreased synthesis due to malnutrition through activation of the complement cascade. Because the decreased C3 was disproportionate to the decrease in C4, it is possible that the alternative pathway was activated as well, leading to increased C3 consumption.

Results from the present study suggest that subjects had secondary C3 and partial C4 deficiencies rather than primary deficiencies. This is because CH_{50} activity was normal, the C4 deficiency was inconsistent and the degree of depression of C3 was not severe in the study subjects. Our subjects would be expected to be at risk for recurrent infection due to the decreased C3 concentrations, but the risk is probably less than in those with severe deficiency due to somewhat higher levels of C3 in our patients.

In conclusion, the present study identified immune abnormalities in children with beta thalassaemia major. The results from this study showed that nutritional factors were important determinants and that these immune abnormalities can be reversed with nutrition support.

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