EVALUATION OF T LYMPHOCYTE SUBSETS IN CHILDREN WITH BETA THALASSEMIA MAJOR

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Abstract - Peripheral blood T lymphocytes and their subsets were studied in 31 patients with beta thalassemia major (age 2-12 years) and compared with 14 age-and sex-matched healthy controls. Three monoclonal antibodies (anti-CD3, anti CD4, anti-CD8) were simultaneously applied for detection of Th ($CD3^+$, $CD4^+$), Ts/c ($CD3^+$, $CD8^+$) and Th/Ts ratio by flow-cytometry respectively. The results of this study showed a slight increase in the number of T lymphocytes, T $CD4^+$, T $CD8^+$, and $CD4^+/CD8^+$ ratio; but this increase was not statistically significant ($P>0.05$). No primary defect in T cell subsets was detected and it was suggested that continuous regulation of iron balance is an important factor in decreasing immunological disturbance.

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Key words: Beta thalassemia major, T cell subsets

INTRODUCTION

Beta thalassemia major (BTM) is a chronic hemolytic anemia, caused by any of approximately 150 mutations within the beta globin gene that results in defective production of beta globin chain of the hemoglobin molecule. Repeated blood transfusions are needed to prevent symptomatic anemia and to keep children alive (6). Thalassemia major affects patients in many ways, some side effects are related to the abnormal hemoglobin synthesis, others are due to the accumulation of iron in the tissues (3,6) and some results from the serious problems due to increased incidence of infections (12). These suggest that a basic defect in the host defense is present in this disorder. Various immunological abnormalities have been considered in polytransfused thalassemic patients, some findings connect these abnormalities to iron overload (3,6) and chronic stimulation by repeated blood transfusions (4). In this study we have analysed T cells and their subpopulations in BTM patients continuously treated with iron chelating agents and blood transfusion regimen.

MATERIALS AND METHODS

Thirty one patients, aged 2-12 years (18 male and 13 female), with beta thalassemia major were studied. All patients had an evidence of disease through clinical examination, abnormal hemoglobin electrophoresis and increased HbF levels. None of them had undergone splenectomy. Each was regularly transfused with packed red cells every 15-30 days in order to maintain Hb>10 g/dl. Fourteen healthy normal sex - and age - matched subjects (2-12 years old), served as controls. None of the patients showed any acute or chronic infectious disease at the time of study. Only beta thalassemia major subjects were included in the study. All of them were receiving desferrioxamine and folic acid. Hematological and immunological studies included complete blood count (CBC), and hemoglobin electrophoresis. Direct immunofluorescent testing for T cell subpopulations was performed, using 3 monoclonal antibodies simultaneously (anti-CD3, anti-CD4, anti- CD8; DAKO-Denmark) to detect $CD3^+$ cells, $CD3^+$ and $CD4^+$ cells and also $CD3^+$ and $CD8^+$ cells by coulter EPICS ELITE flow cytometer.

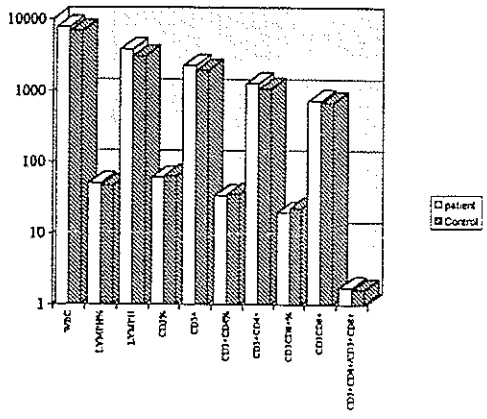

Fig. 1. Comparision of different factors between patients and controls

RESULTS

Patients and controls were divided into subgroups by age and sex. As the leukocyte percentages have sharp changes according to age, we had chosen two subgroups ranging from 2-7 years and 8-12 years for patients and controls as well. Age can also influence the effects of treatment and the duration and amount of transfusion.

Patients were also categorized in terms of hepatosplenomegaly. Comparing these two patient groups, we were able to see the outcome of liver and spleen abnormalities. Using WBC count and lymphocyte percentage, the absolute counts were calculated from the percentage obtained by flow cytometry. Ten parameters between the subgroups were compared by Student t-test; WBC count, lymphocyte percentage and absolute count, T lymphocyte percentage, and absolute count, T helper percentage and absolute count, Ts/c percentage and absolute count and $CD4^+/CD8^+$ ratio. As shown in Table 1 and Fig. 1, a slight increase in the mean number of leukocytes, lymphocytes, T cells, T-helper cells, T-cytotoxic cells and CD4/CD8 ratio of patients were determined and compared with matched control subgroups. The mean number of T-helper cells were increased, but using Student t-test (Table 2), elevations in all absolute counts were not statistically significant ($P>0.05$).

DISCUSSION

The most common problem in patients with BTM is iron overload that is caused by repeated blood transfusions. Cellular iron homeostasis is essential for a variety of vital processes, growth and also regulation of immune function. There are some reports about the linkage of cell-mediated immunity (CMI) to iron metabolism (1,2,12,14). In fact both iron overload and iron deficiency can influence the immune status by altering the proliferation of T and B cells. The cellular iron may affect the proliferation of TH_1 and TH_2 subsets, thus iron has a role in modulating the activities of T cell subpopulations and consequently the immune effector mechanisms (14).

Furthermore, excess iron has an influence on CMI which plays a major role in host defence against intracellular pathogens. Interferon (IFN) gamma secreted by TH_1 cells, activate macrophages to produce reactive oxygen species and enzymes to kill phagocytosed pathogens. Several reports have demonstrated that iron - laden macrophages lose the ability to kill intracellular pathogens (1,14). Production and activity of tumor necrosis factor alpha (TNFα) is decreased in iron - laden macrophages while adjustment of iron by desferrioxamine therapy upregulates TNF- Rs.

Table 1. Mean and SD of patients and controls

Groups	LYMPH WBC /μl	LYMPH %	LYMPH /μl	CD_3^+ %	CD_3^+ /μl	$CD_3^+\&CD_4^+$ %	$CD_3^+\&CD_4^+$ /μl	$CD_3^+\&CD_8^+$ %	$CD_3^+\&CD_8^+$ /μl	$CD_3^+\&CD_4^+/CD_3^+\&CD_8^+$ %
All	7625.8	49.9	3758.9	59.9	2231.1	33.5	1254.4	19.6	731.7	1.72
Patients	2394.4	10.1	1245.2	9.4	1804.9	6.9	498.9	3.1	258.5	0.39
Patients	7862.5	49.5	3854.5	61.6	2376.8	34.1	1316.3	19.8	768.6	1.75
with organomegaly	2004.2	9.3	1207.1	8.5	842.6	6.7	494.6	3.3	295.3	0.43
Patients	7373.3	50.3	3657.1	58.1	2075.6	32.9	1183.3	19.5	692.3	1.69
without organomegaly	2801.6	11.1	1319.1	10.3	759.9	7.2	212.1	3.1	215.8	0.35
Patients	7673.3	53.4	4151.8	59.6	2444.1	33.6	1377.1	19.4	799.6	1.76
under 8	1908.1	9.51	1422.9	9.8	911.4	7.51	546.1	3.5	314.6	0.46
Controls	7071	51	3496.5	61.2	2160.8	32.8	1156	22.1	779.1	1.52
under 8	2132.1	8.1	750.1	8.1	592.8	8.6	379.4	3.2	235.8	0.50
Patients	7581.2	49.5	3390.6	60.1	2031.5	33.5	1139.4	19.9	667.9	1.69
above 8	2835.7	9.4	955.3	9.4	657.8	6.5	436.2	2.8	179.8	0.32
Controls	6557.1	40.8	2557.7	66.2	1692.1	37.8	972.8	22.6	579	1.72
above 8	1625.6	10.3	336.1	8.2	305.8	5.3	223.6	4.8	135.6	0.34
	7672.2	50.4	3829.2	58.5	2227.9	33.1	1259.5	19.3	746	1.72
Male patients	2572.6	11.1	1342.7	10.5	916.3	7.7	543.4	2.9	311.9	0.41
	6384.5	45.7	2809.2	61.6	1726.5	32.1	892.6	22.4	632.6	1.5
Male controls	1887.1	10.3	629.7	9.2	475.8	7.2	247.1	4.9	229.1	0.52
	7561.5	49.1	3661.6	61.9	2235.5	34.1	1247.3	20.1	711.9	1.73
Female patients	2224.6	8.5	1142.4	7.7	655.8	5.6	451.5	3.5	168.9	0.36
	7383.3	46.1	3317.6	66.6	2193.1	39.6	1293.5	22.2	737.5	1.78
Female controls	1773.6	11.3	833.8	6.3	470.6	5.2	246.3	2.5	191.3	0.19
	6814.2	45.9	3027.1	63.7	1926.5	35.3	1064.4	22.3	677.5	1.62
All Controls	1840.9	10.3	714.1	8.2	514.3	7.3	313.9	3.9	212.7	0.42

Table 2. Comparison of different groups of patients and controls by Student t-test

Comparison of different groups by student t.test	WBC /μl	LYMPH %	LYMPH /μl	CD_3^+ %	CD_3^+ /μl	$CD_3^+\&CD_4^+$ %	$CD_3^+\&CD_4^+$ /μl	$CD_3^+\&CD_8^+$ %	$CD_3^+\&CD_8^+$ /μl	$CD_3^+\&CD_4^+/CD_3^+\&CD_8^+$ %
Patients and Controls	P=.267	P=0.229	P=0.048 P<0.05	P=0.199	P=0.202	P=0.447	P=0.198	P=0.021 P<0.05	P=0.497	P=0.415
	NS	NS	S	NS	NS	NS	NS	S	NS	NS
Patients without (no organornegaly) and controls	P=0.149	P=0.329	P=0.035 P<0.05	P=0.500	P=0.094	P=0.661	P=0.113	P=0.075	P=0.347	P=0.401
	NS	NS	S	NS	NS	NS	NS	NS	NS	NS
Patients (and organomegaly) with controls	P=0.534	P=0.278	P=0.128	P=.116	P=0.544	P=0.388	P=0.443	P=0.040 P<0.05	P=0.845	P=0.601
	NS	NS	NS	NS	NS	NS	NS	S	NS	NS
Patients without (organomegaly) and patients (with organomegaly)	P=0.578	P=0.821	P=0.667	P=0.303	P=0.306	P=0.625	P=0.485	P=0.748	P=0.421	P=0.688
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Patients under 8 years and controls under 8	P=0.514	P=0.564	P=0.269	P=0.713	P=0.464	P=0.830	P=0.348	P=0.112	P=0.880	P=0.275
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Patients above 8 years and controls above 8	P=0.386	P=0.210	P=0.037 P<0.05	P=0.155	P=0.210	P=0.145	P=0.354	P=0.111	P=0.242	P=0.848
	NS	NS	S	NS	NS	NS	NS	NS	NS	NS
Patients under 8 years and patients above 8	P=0.917	P=0.053	P=0.089	P=0.885	P=0.157	P=0.980	P=0.190	P=0.658	P=0.160	P=0.592
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Controls under 8 years and controls above 8	P=0.621	P=0.064	P=0.011 P<0.05	P=0.275	P=0.088	P=0.218	P=0.293	P=0.798	P=0.072	P=0.412
	NS	NS	S	NS	NS	NS	NS	NS	NS	NS
Male patients and male controls	P=0.219	P=0.323	P=0.053	P=0.480	P=0.160	P=0.739	P=0.082	P=0.061	P=0.367	P=0.253
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Female patients and female controls	P=0.866	P=0.529	P=0.520	P=0.210	P=0.889	P=0.061	P=0.819	P=0.215	P=0.772	P=0.752
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Female patients and male controls	P=0.336	P=0.944	P=0.217	P=0.277	P=0.093	P=0.053	P=0.011 P<0.05	P=0.931	P=0.383	P=0.230
	NS	NS	NS	NS	NS	NS	S	NS	NS	NS
Female patients and male patients	P=0.901	P=0.730	P=0.718	P=0.332	P=0.980	P=0.713	P=0.948	P=0.538	P=0.724	P=0.948
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

S = Significant NS = not Significant

In this study, T cells andtheir subpopulations were evaluated in patients with beta-thalassemia major. A number of investigators studied cell mediated immunity, but results are controversial. The total number of circulating T lymphocytes in patients were found to be normal (8, 9) or decreased (4, 7, 10). In some studies a reduction in the number of T helper cells was observed resulting in a decreased Th/s ratio (3, 5, 7, 11, 12, 15). In other patient groups, analysis of lymphocyte subsets was normal (1,11). Another report has pointed out a slight increase in the number of T lymphocytes and T helper cells (13). In our patients who were continuously treated with the iron chelating agent desferrioxamine, slight increase in the number of leukocytes, lymphocytes, T cells, Ts cells, CD4/DC8 ratio and especially Th cells were observed, but these changes were not statistically significant ($P>0.05$). The results of this study showed no primary defect in T cell subsets and support the view of association between iron overload and T cell subset abnormalities, and suggest that contiuous regulation of iron balance is an important factor in decreasing immunological disturbance.

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