Characteristics of "Early Type-I Pattern" (0-6 years) Hodgkin's Disease in Turkish Children

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ABSTRACT

The clinico-epidemiologic characteristics of 54 children with HD in 0-6 years of age group were retrospectively analyzed. This group represented 27% of 200 HD cases observed in our center and was named as early type-I pattern HD. The association of EBV with HD was also shown by serologic and immunohistochemical methods (LMP_1) in these very young Turkish patients. T-cell immune deficiency, cytokine imbalance and Zn deficiency were additional findings found in these patients. This series seems to be the largest one studying early type-I HD, by several aspects.

Key Words: Type-I pattern Hodgkin's disease, Turkish children.

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Several epidemiologic studies on Hodgkin's disease (HD) revealed three patterns attributable to interactions of environmental and host factors[1,14]. The type-I pattern is characterized by a high incidence of mixed cellular (MC) and lymphocyte depletion (LD) histologic subtypes in children of developing countries, whereas type III shows a pronounced peak in young adults and a predominance of the NS histologic subtype in developed countries[1,14-16]. The intermediate type II is observed in rural areas of developed countries.

Our previous retrospective analysis of 175 children with HD, under 16 years of age, revealed a developing country or type-I pattern. Even interestingly, this pattern occurred even in children of 0-6 years of age group[16]. Therefore, we named

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this group as early type-I pattern HD. However, data in the literature regarding these very young children with HD appear to be rather limited. The purpose of this study is to analyze further clinical and laboratory characteristics of early type-I HD in detail.

MATERIALS and METHODS

Fifty-four cases under 6 years of age were observed among a series of 200 children with HD who were diagnosed with surgical tissue biopsy between 1964 and 2001 in Pediatric Oncology-Hematology Research Center of Ankara University.

We retrospectively analyzed epidemiological, clinical and histologic features of the 54 patients in this report. The main parameters taken into consideration were the age, sex, clinical stage and histologic subtypes of the patients. Ann-Arbor modification of the Rye system was used for clinical staging. Physical examination, blood counts, bone marrow examination, basic biochemical and radiological studies have been obtained for staging. Imaging techniques (abdominal ultra sonography, chest and abdomen computerized tomographic scan) have been used during the recent years[14,16].

EBV studies: Frequency of EBV infection was first studied serologically in 324 healthy children between 6 months and 15 years of age for IgG antibodies against EBV-viral capsid antigen (VCA). In 51 patients with HD anti-VCA antibodies were also determined by indirect immunofluorescence method[16,17].

EBV Latent Membrane Protein (LMP-1)

A total of 34 initial biopsy samples obtained from the patients with HD below 16 years old for the study of EBV-LMP_1. Five of them were under 6 years of age. Paraffin sections were stained with monoclonal antibodies by immunuhistochemical method as described before[16,18].

T-Cell Immunity

T-cell immunity was evaluated by the T-cell subpopulation of CD4, CD8 and natural killer (CD16) cells, measured by monoclonal antibodies. Delayed hypersensitivity reactions to PPD antigen, as well as lymphocyte proliferative response (LPR) to PHA were also determined in a variable number of patient with a range of 6-11[15,16].

Trace elements, mainly serum and plasma zinc (Zn) levels in addition to erythrocyte and hair Zn concentrations and serum copper (Cu) levels were measured in patients ranging in number from 7 to 25, by atomic absorption spectrophotometry before therapy[15,16,19,20].

Cytokines

Serum cytokine levels were recently studied in 23 patients with HD[22a,22b]. Six patients below 6 years of age, were included in this study. Soluble IL-2R, IL-2, IL-4 and tumor necrosis factor-alfa (TNF-a), were measured in a sandwich Elisa technique[21].

RESULTS

The general characteristics of 54 patients including age, sex, clinical stage and histology with early type-HD are summarized in Table 1. This group represented 27% of total 200 HD patients in our series. The majority of the cases were male

Table 1. Early type-I (0-6 years)-Hodgkin's disease (Pediatric Oncology, Ankara-University)

		Percentage	Total no
	54	27	200
Sex	M: 41	76	
	F: 13	24	
	M/F: 3/1		
Clinical stage x			
St I-II	21	42.9	
St III-IV	28	57.1	
Histopathology x			
LP	10	20.8	
NS	9	18.7	
MC	29	60.4	
LD			

49 patients were included.

(76%) even in this early group, with a male to female ratio of 3:1. Analysis of clinical stages revealed that advanced stages (stages III and IV) predominated (57.1%) in the patients. The distribution of histologic subtypes showed a relatively high frequency of MC type accounting for 60.4% of the patients. Almost all children with HD in this age group were from low SES. These characteristics were compatible with type-I epidemiological pattern which we named as early type-I HD in Turkish children. Distribution of sex, clinical stages and histologic subtype were essentially very similar to those found previously in the group of 175 patients with HD[16]. The distribution of early HD was reviewed through the reports in other pediatric oncology centers[24-26]. Three hundred thirteen patients below 6 years of age, out of total 1027 HD cases were found in 6 pediatric oncology centers/units within Turkey (Table 2).

Incidence of EBV infection measured by anti-VCA-IgG antibodies, according to the age in 324 control children, is shown in Table 3. Seropositivity of 57.5% to EBV was found even in 6 months to 6 years old healthy children. Highest percentage of exposure was noted in 7-10 years age group with 81.8% seropositivity. The incidence of EBV infection studied in 51 HD patients are shown in Table 3a. Seropositivity was found to be 94% in the whole group of patients and 100% in the early type-HD subgroup of 2.5 to 6 years of age. These values were significantly higher as compared to that of controls (71.7%).

Table 3. Incidence of EBV infection according to age in control children

Age	n	Positive (percent)
6 months-6 years	75	57.5
7-10 years	132	81.8
11-15 years	117	76.0
Total	324	71.7

Table 3a. Incidence of EBV infection in children with Hodgkin's disease

	Hodgkin	
	Total	Positive
AGE groups	n	%
2.5-16 years	51	94%
	Early type-I HD	
2.5-6 years	12	100%
Control	324	71.7%

p< 0.01

Table 2. Early type-I (0-6 years) Hodgkin's disease in 6 centers in Turkey

	Pediatric Oncology Centers				
	Early type-I HD		Total HD		
	n	Percentage	n	Years	Authors
1. Ankara University	54	27	200	1965-2001	Çavdar, et al*
2. Sami Ulus Children's Hospital	43	25.0	175	1985-2001	Ertem U, et al**
3. Cerrahpaşa Med School	21	46.6	45	1977-1995	Yıldız I, et al
4. İstanbul University	10	22	45	1989-1990	Ayan I, et al
5. Gazi University	9	20	46	1991-1995	Oğuz A, et al
6. Hacettepe University	176	34	516	1973-1992	Çevik N, et al
Total	313	29.1	1027		

* Present series,
** Unpublished data.

The results of the EBV-LMP_1 study in 34 pediatric HD patients, was demonstrated in Table 4. Twenty-two (64.7%) cases were found were positive for LMP_1 expression. Three out of 5 patients under 6 years of age were positive for LMP_1, in this group. T-cell immunity evaluated by several parameters was shown in Table 5. The mean CD4 and CD8 cells counts were found to be significantly lower than the controls while CD16 was higher than normal. Ninty percent of the patients were anergic to PPD and LPR was also low as compared to controls. Zn status, including RBC, hair Zn levels and serum Zn concentrations are shown in Table 6. All Zn values were significantly low as compared to control children, a finding parallel to our earlier results found in HD. This reflects chronic Zn deficiency in these young children while serum Cu levels were elevated prior to therapy[16,19,20].

Results of the serum cytokine analysis of patients with HD in the early type-I group revealed

Table 4. EBV-LMP_1 in "Pediatric Hodgkin" disease

No	Age (years)	Sex	Histology	LMP, (+) No	(+)
34	4-16	26 M	14 MC,16 NS	22	64.7%
		18 F	4 LP		
			Early age group		
5	4-6	4 M	4 MC	3	60%
		1 F	1 NS		

Table 5. T-cell immunity in pediatric Hodgkin disease

	T-cell subsets (monoclonal study)		
T-cell subgroup	Early group	Control	p
CD4 (%)	25.9 ± 8.4	33.7 ± 1.8	< 0.001
	(6)*	(25)	
CD8 (%)	23.1 ± 1.6	29.1 ± 1.8	< 0.01
	(6)	(25)	
CD16(%)	19.7 ± 1.6	14.8 ± 1.9	< 0.005
	(5)	(20)	
	Other cellular immune tests		
	Early HD (Patients)	Control	p
DHR**			
Anergy (%)	90%	0%	
(PPD)	(11)	(20)	
LPR***	38.6 ± 4	70.1 ± 2.4	< 0.005
	(6)	(20)	

* Number in parenthesis indicates no of studies cases,
** DHR: Delayed hypersensitivity reaction (in older children > 6 years to antigens PPP and DNCB were tested),
*** LPR: Lymphocyte Proliferative Response to Phytohemagglutinin.

Table 6. Zinc (Zn) status* and S. copper (cu) in early Hodgkin's disease

	Zinc status				
	Plasma Zn (µg/dL)	Serum Zn (µg/dL)	RBC Zn (µg/dL)	Hair Zn (µg/dL)	Serum Cu (µg/dL)
HD	63.3	73.3 ± 16.4	10 ± 1.1	99.5	196.3 ± 47.3
n	(7)	(13)	(7)	(25)	(13)
Control	109.8 ± 12.3	110.5 ± 15	15.8	184.0 ± 19	161.0 ± 4
n	(83)	(20)	(34)	(97)	(20)
p	< 0.001	< 0.01	< 0.01	< 0.001	

* Chronic zinc deficiency.

higher levels of IL-2R, IL-2 and TNF-a levels when compared to normal children Table 7.

DISCUSSION

HD is rare in children below 5 years of age in Western countries. This lymphoma presents a definite bimodal age specific peaks, in developed world. The first peak appears in the 15 to 40 years age group and the second peak occurs after the age of 50, whereas the early peak appeared before adolescence in developing countries[6,8,16,22].

The retrospective analysis of 54 children with HD under 6 years of age in this study revealed a "developing country pattern" or type-I pattern. Since this pattern occurred in early childhood (0-6 years of age), we named this group as an "early type-I". Although type-I pattern of HD under 15 years of age has been reported in children by several developing countries, there seems to be no large enough study in very young children with HD (0-6 years of age) in the literature[1,6,9-16,23-29]. In fact, analysis of age distribution of 2.163 consecutive patients with HD from Stanford University-Medical Center, revealed that only 4% occurred below 10 years of age group, whereas in Turkish series nearly one third of all HD patients fell within the age group under 6 years[30]. Earliest age of HD was 1 year in 2 centers and 2 years in two others including our center, in Turkey.

Several studies suggested that the histologic type of HD is strongly influenced by the interaction between host and environmental factors, although clinicoepidemiologic and histologic heterogeneity of pediatric HD is probably due to multifactorial effects of a complex process[1-3,16,31-37]. Environmental factors are likely to be more important than racial factors in controlling the frequency and type of lymphoma, according to several reports[1-3,6,18,19,26,33]. Low SES and poor nutrition are important factors because of their adverse effects on the immune system[1,6,19,26-28]. The age-related acquisition of antibodies to EBV is a useful marker of SES of a given population. The peak time for seroconversion occurs during adolescence in economically advanced populations. However, healthy Turkish children developed anti-EBV (VCA) antibodies even at 1 year of age, reflecting early exposure to EBV[15-17].

Table 7. Serum cytokine levels in early Hodgkin's disease 0-6 years

Cytokine	Early type-I HD (n= 6)	Control (n= 10)	p
sIL-2R	3916.6 ± 2035	557 ± 125	< 0.0001
IL-2	404.1 ± 1.43	220 ± 108	< 0.05
IL-4	12.5 ± 3.2	25 ± 17	NS*
TNF-a	856.6 ± 565	35 ± 16.7	< 0.005

* NS not significant.

Moreover, EBV-related LMP_1 positivity has been shown to be in 64.7% of 34 pediatric HD patients in this study, and 60% in the "early age" group (below 6 years), (Table 4). The high percentage of LMP_1 positivity in our series indicates the potential pathogenetic effect of EBV[16,29,32]. LMP_1 expression in the studied Turkish children with HD is higher than that reported in Western countries, but is similar to that described in studies from other developing countries[4,5,15-17,27,32,33]. The presence of LMP_1 indicates that EBV is not merely a silent passenger but may be involved in the pathogenesis of HD since it has an oncogenic property[29,32-34]. There is already substantial epidemiologic, serologic, and molecular biologic evidence that EBV is involved in the pathogenesis of pediatric HD[4-6,15,16,29,31-38]. The association of EBV with HD in a large international case series suggested the involvement of EBV in the pathogenesis of HD and confirmed that the association was strongly linked to histologic subtype[32]. The distribution of EBV associated HD in young children (10 years or younger) is not random and according to the reports that HD is, predominantly an EBV associated entity in children[5,29,31,32]. Our findings of association of EBV with HD in early age group is compatible with this information. It is also known that immunodeficiency states (hereditary or acquired) predispose to lymphomas including HD[36-40]. Our previous study revealed diminished T-cell functions including anergy associated with Zn deficiency in the children with HD below 16 years of age[16,19,20]. CD16 in some of the early cases of HD. In this study CD4 and CD8 expressions were significantly low in the youngn patients, in addition to anergy and decreased LPR. Elevated serum soluble IL-2 receptor (sIL-2R) and TNF-a levels were also observed in this group of patients with HD. It is known that malignant cells produce several cytokines in HD, which are probably responsible for HD's many clinical and laboratory findings, including immunodeficiencies[21,39].

The correlation between Zn deficiency and reduced immune functions has been reported in several clinical conditions[19,20, 40,41]. Therefore, we believe that in Turkish children with HD a number of factors, such as "nutritional environment" associated with chronic Zn deficiency and cytokine imbalance, may have conributed to the impaired cellular immunity[6,14,16,19,20,21,39].

In conclusion, the retrospective analysis of 54 patients with HD in children of 0-6 years of age group, revealed the characteristics of type-I pattern, named as early type-I HD (Table 8). Review of the cases in other pediatric oncology centers also revealed that nearly 1/3 of HD patients fell into this group in Turkey. Therefore, this report reflects one of the largest group of HD in very young children in Turkey. The high frequency of EBV association with HD was also shown by serological and immunocytochemical methods in the early childhood HD patients. We believe that early type HD represents a biologic model for studies from different aspects. Further studies on environmental, viral factors and molecular changes are needed. Future use of vaccine against EBV may prevent early EBV infection and possibly subsequent development of HD in Turkish children.

Table 8. Characteristics of "early" type-1 Hodgkin's disease

Early age: 0-6 years
Male predominance
Advanced clinical stage (St- III-IV)
MC histology (predominates)
Early exposure to EBV
Low SES

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