Hodgkin's disease and association with Epstein–Barr virus in children in Southeast Turkey

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ABSTRACT

Objectives: There are relatively few reports on histologic and immunophenotypic features of Hodgkin's disease (HD) in children in Turkey. The aim of the present study is to characterize the clinicopathologic, immunophenotypic features and Ebstein-Barr virus (EBV) status of HD in children in our region.

Methods: Fifty-two cases coded as HD in the Department of Pathology, Medical Faculty, Dicle University, Turkey, from 1990 to 2002 were retrieved. We analyzed clinicopathologic data, immunophenotype, and EBV status of all patients.

Results: In most cases, the cervical lymph nodes were involved. The 52 cases (35 boys, 17 girls; male to female ratio was 2.1) were categorized as mixed cellularity (MC) in 23 (44.2%), lymphocyte predominance (LP) in 16 (30.8%), nodular sclerosis (NS) in 10 (19.2%), and lymphocyte depletion (LD) in 3 (5.8%). Of 33 cases, including MC and NS, Hodgkin's Reed-Sternberg cells and variants were positive for CD15 in 27 cases (81.8%) and positive for CD30 in 30 cases (90.9%). All the cases of LP demonstrated the characteristic phenotype of this variant (CD45RB+, CD20+, CD15-, CD30-). Epithelial membrane antigen stained the lymphocytic/histiocytic cells in 3 cases (18.7%) of 16. Ebstein-Barr virus-LMP1 were positive in 31 cases (61.5%) of a total of 52 cases and the most were MC (91.3%).

Conclusion: In conclusion, mixed cellularity HD is the most common histologic subtype. This result differed from that reported in developed countries. We found a striking association with expression of EBV-latent membrane protein 1 in malignant cells in childhood HD.

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Hodgkin's disease (HD) is a clinically aggressive disease of the lymphoid tissues and usually fatal if left untreated. Traditionally, the nature of HD has been controversial, in particular whether it is a primarily infective or a neoplastic process. Although certain clinical, histopathologic, and epidemiologic features are reminiscent of an infection, the disease is regarded as a malignant lymphoma.[1] Hodgkin's disease, which typically has a bimodal distribution, has been reported to be more common in children from developing countries than in North American and European children.[2] In developing countries, the first age peak occurs between the ages of 7 and 12 years, whereas in industrialized countries, the initial peak is delayed until early adulthood.[3,4] It has been suggested that differences in the age at which someone is exposed to an infective agent, possibly Epstein-Barr virus (EBV) might be responsible for these epidemiologic features.[5,6] Although there is a large body literature describing the pathology of HD in adults, there are relatively few reports in pathology literature that characterize the histologic and immunophenotypic features of HD in children. The aim of the present

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study, therefore, is to characterize the clinicopathologic, immunophenotypic features and EBV status of HD in children from Turkey, which includes Southeast Anatolian children from a poor rural environment.

Methods. Fifty-two cases of HD in Turkish children aged ≤15 years were selected on the basis of the availability of paraffin blocks and complete clinical records. Clinical records were reviewed and following data were recorded: age, gender, staging of disease, histopathologic subtypes at diagnosis and presence of B symptoms. The clinical and pathological staging was accomplished according to the Ann Arbor system.[7] Staging procedures included clinical history, physical examination, chest x-ray, liver-spleen scans, computed tomography, or ultrasound of the abdomen and pelvis. All cases were nodal. All tissues were formalin-fixed and paraffin-embedded. Hematoxylin and eosin stained sections were re-analyzed histologically, and cases were classified according to the Rye-classification.[8]

For immunohistochemistry, all primary and secondary antibodies were from Dako (Glostrup, Denmark). Paraffin sections were cut onto silanized slides and immunohistochemical staining was performed according to the standardized avidin-biotin complex methods. The antibody panel for each case included CD45RB (leukocyte common antigen), CD15 (Leu M1), CD20 (L26), CD30 (Ber H2), epithelial membrane antigen (EMA) and CD3. In addition, all cases were stained with the antibody EBV-latent membrane protein 1 (EBV-LMP1). Positive and negative controls were used throughout.

Results. **Table 1** summarizes the available clinical data for HD cases. There was a male predominance. Of the 52 cases, there were 35 boys and 17 girls, with a male to female ratio of 2:1. The youngest patient was a 4-year-old boy. The number of cases increased with advancing age, which resulted in a mean age of 10.3 years.

In most cases, the cervical lymph node (71.1%) was the anatomic location most frequently involved. Other common sites were axillary (13.5%), mediasten (7.7%), abdomen (5.8%) and inguinal lymph nodes (1.9%) (**Table 1**). The distribution of HD cases with regard to histologic subtypes and clinical stages are given in **Table 2** Overall, the mixed cellularity (MC) subtype was the most common, accounting for 44.2% of the cases, followed by the lymphocyte predominance (LP) subtype with 30.8%. Only 19.2% of cases showed nodular sclerosis (NS) HD, and there were 5.8% cases of lymphocyte depletion (LD) subtype. Mixed cellularity cases contained numerous Hodgkin's Reed-Sternberg (HRS) cells in an inflammatory cellular background, which is the characteristic of HD. The HRS cells showed a variety of morphologies ranging from classic variants, to anaplastic atypical multinucleated tumor giant cells, to immunoblast-like cells. Some cases showed diffuse effacement of lymph node architecture with the classic appearance of MC subtype. In other cases, however, the tumor that infiltrates had a vaguely nodular architecture.

Of all cases, 21 had systemic symptoms such as, fever, night sweating and losing weight. When the stage of cases had raised, the B symptoms also increased. There was a clear correlation between histologic subtypes, stages and the B symptoms. The correlation between histologic subtypes, stages and the B symptoms were presented in **Table 3**.

The immunohistochemical results and EBV-LMP1 status of our cases are summarized in **Table 4**. Virtually, MC, NS and LD subtypes, the immunophenotype of neoplastic cells were CD30+, CD15+, and negative for T-cell markers. Including cases of MC and NS subtypes, RS cells and variants were positive for CD15 in 27/33 cases (81.8%) and positive for CD30 in 30/33 cases (90.9%). In 3/33 cases (9.0%), RS cells expressed CD20, whereas none of cases showed reactivity for CD3. All the cases of LP-HD demonstrated the characteristic phenotype of this variant (CD45RB+, CD20+, CD15-, CD30-). Of 3 LD cases, one case was positive for CD15 and 2 cases were positive for CD15. Epithelial membrane antigen stained the lymphocytic/histiocytic cells in 3/16 cases (18.7%). Epstein-Barr virus-LMP1 were positive in 31/52 (61.5%) of cases and most of them were MC (91.3%).

Discussion. The main objective of this retrospective analysis was the comparison of results in childhood HD (CHD) at our region with the

Table 1 - Clinical and demographic characteristics of our cases.

Characteristic	Patients n	(%)
Gender		
Male	35	(67.3)
Female	17	(32.7)
Age (years)		
4-6	4	(7.7)
7-9	17	(32.7)
10-12	14	(26.9)
13-15	17	(32.7)
Location		
Cervical	37	(71.1)
Axilla	7	(13.5)
Mediasten	4	(7.7)
Abdomen	3	(5.8)
Inguinal	1	(1.9)

Table 2 - Distribution of histologic subtypes and clinical stages according to the age groups.

Characteristic	N of patients 4-6	7-9	10-12	13-15	Total N (%)
Histopathologic types					
Lymphocyte predominance	1	5	3	7	**16 (30.8)**
Mixed cellularity	1	9	8	5	**23 (44.2)**
Nodular sclerosis	2	1	3	4	**10 (19.2)**
Lymphocyte depletion	-	2	-	1	**3 (5.8)**
Clinical stage					
I	1	5	7	4	**17 (32.7)**
II	2	4	3	5	**14 (26.9)**
III	1	5	3	6	**15 (28.8)**
IV	-	3	1	2	**6 (11.6)**

Table 3 - Distribution of the histologic subtypes according to stages and systemic symptoms status.

Subtypes	Stage I	II	III	IV	B symptoms
Lymphocyte predominance	10	4	2	-	4
Mixed cellularity	5	8	8	2	9
Nodular sclerosis	2	2	3	3	6
Lymphocyte depletion	-	-	2	1	2
Total	**17**	**14**	**15**	**6**	**21**
B symptoms	1	5	11	4	-

Table 4 - Immunophenotypic results and EBV-LMP1 status of 52 cases.

Subtypes	CD15	CD30	CD45RB	CD20	EMA	CD3	EBV-LMP1
Lymphocyte predominance	0/16	0/16	16/16	16/16	3/16	0/16	6/16
Mixed cellularity	18/23	22/23	2/23	1/23	0/23	0/23	21/23
Nodular sclerosis	9/10	8/10	0/10	2/10	0/10	0/10	4/10
Lymphocyte depletion	1/3	2/3	1/3	0/3	0/3	0/3	1/3

EBV-LMP1 - Ebstein-Barr virus-latent membrane protein 1, EMA - epithelial membrane antigen,

results in other parts of the world. As regards to the relative frequency of malignant lymphomas in children, the incidence of HD is higher in underdeveloped countries than non-Hodgkin's lymphoma.[9] Generally, HD is a less frequent lymphoma in most developing countries, compared with Western Europe and North America. Hodgkin's disease accounts for some 7.9-18% of all lymphomas in China,[1,10] whereas some studies from Western countries report rates of up to 51%.[10,11] Hodgkin's disease accounts for between 5.3% and 11% of childhood cancers in Africa.[12] Similar to previous reports from both developed and developing countries.[2,13] However, a different epidemiological picture is seen with pediatric HD.

The first peak in the bimodal age-incidence curve of HD, seen in the third decade in developed countries, occurs earlier in developing countries.[1] The youngest patient with documented HD in the literature was a 5-month-old boy with thymic alymhoplasia.[14] The mean age of CHD in underdeveloped countries is lower than in developed ones, probably as those countries have a larger infantile population.[1,15] In our study, the mean age was 10.3 years. Most of our cases were males with a male to female ratio of 2:1 and the youngest patient was a 4-year-old boy. The clinical presentation, as expected, was about three-quarters of the children presenting with cervical lymphadenopathy. Similar findings have been reported by other studies.[1,2,9,14,15]

Different epidemiological patterns of HD have been described.[12,16] Type I HD is characterized by high rates in children and predominance of MC or LD subtypes in population with poor socio-economic conditions. Type III HD has an initial age peak in young adults with very low rates in children and a predominance of NS subtype in wealthy urbanized countries. Type II HD has an intermediate pattern. Children from a poor socio-economic background, who are exposed to other environmental factors had developed HD at an earlier age and the distribution of histological subtypes is dissimilar to that of children from a good socio-economic background.[12] Similarly, our cases had similar characteristics that of type I HD.

In our study, the MC subtype has been seen more frequently in children such as other developing countries and the second most frequent subtype is LP. The findings from other pediatric oncology centers in Turkey and other developing country supports that the age and histologic distributions are similar to our findings.[1,15,17,18] Although the pathogenesis of HD is unknown, in particular the variation in incidence seen in different age, gender, ethnic, geographic, and socio-economic groups suggest that different environmental etiologic agents (such as one or more common viruses) may be involved.[1,4] The EBV has emerged as the leading candidate for such an etiologic agent. Initially, this was the result of indirect evidence from epidemiologic and serologic studies.[5,19] Recently, more direct evidence came from the demonstration of EBV nucleic acids and antigens in HD tumor tissues and specifically in HRS cells.[20,21] Mixed cellularity predominance in this study population may explain some viral infections seen at high frequency in our country, such as EBV.[17] Epstein-Barr virus has been implicated ever since it was found that risk of HD was increased following infectious mononucleosis and that persons with HD had raised anti-EBV titers compared with healthy controls.[22] The presence of EBV may correlate with histologic subtypes of HD. For example, EBV is present at a higher percentage in MC and LD histologic subtypes than in NS and LP.[17,22,23] In a Turkish study, EBV-LMP1 and EBV-DNA has been shown high positive in malign cells in children with HD.[24] These findings support the view that EBV is involved in the origin or pathogenesis of MC subtype in HD.[17] In our study, EBV-LMP1 were positive in 31/52 cases (61.5%) of cases and the most of them were MC (91.3%). For this reason, in children, EBV may be cofactor in pathogenesis of HD particularly in MC histologic subtype. Jarrett et al[23] reported that HD in children and adults aged ≥50 years is more frequently associated with EBV, as opposed to young adults. Armstrong et al,[25] in a study of 55 cases of pediatric HD from 3 geographic locations (United Kingdom, Brazil, and Saudi Arabia) concluded that HD in children, particularly in those <10 years of age, is predominantly an EBV-associated disease and did not find a significant difference in EBV association by geographic location. However, other studies showed a much higher prevalence of EBV in pediatric cases from developing countries compared with studies from the United States and Europe. For example, in 2 reports from Peru and Kenya,[13,26] EBV was detected in 94% and 100% of the CHD. The majority of our patients had an early-stage disease in contrast to the ones reported from many developing and even from developed countries.[9,17,27] The frequency of B symptoms was also higher in patients with advanced stages, as expected.

In conclusion, in this study, MC HD is the most common histologic subtype. This result differed from that reported in developed countries. We found a striking association with expression of EBV-LMP1 in malignant cells in the CHD. It seems that factors other than socioeconomic profile and age distribution influence the relationship among HD development, its morphologic features, and EBV infection.[18]

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