HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISTICS OF HODGKIN'S LYMPHOMAS FROM A SINGLE CENTER IN SOUTHEASTERN TURKEY

SEZEN KOÇARSLAN[1], MUHAMMET EMIN GULDUR[1], TURAN EKINCI[2], HASAN ILYAS OZARDALI[3], VEHBI ERÇOLAK[4], EMEL YIGIT KARAKAS[4], METE KOKSAL[5], TURGAY ULAS[4]

[1]Harran University, Faculty of Medicine, Department of Pathology, Sanliurfa - [2]SanlıurfaTraining and Research Hospital, Department of Pathology, Sanliurfa - [3]Kocatepe University, Faculty of Medicine, Department of Pathology, Afyon - [4]Harran University, Faculty of Medicine, Department of Internal Medicine, Sanliurfa - [5]Harran University, Faculty of Medicine, Department of Hystology and Embryology, Sanliurfa, Turkey

ABSTRACT

***Aim**: The aim of the present study was to investigate the clinical, histopathological and immunohistochemical characteristics of Hodgkin's lymphoma (HL) in the city of Sanliurfa in Turkey.*

***Methods**: Thirty-two cases that had been diagnosed as HL and of which complete clinical and histopathological information could be fully accessed at our hospital clinics between 2002 and 2013 were included in the present study. The clinical, histopathological and immunohistochemical findings were retrospectively examined.*

***Results**: The male/female ratio was 2.2. The average age of the subjects was 29.9±24.4. The distribution of the ages of these cases, in terms of decades, was 31.3% in the first decade, 9.4% in the second decade, 21.9% in the third decade, 6.3% in the fourth decade and 28.1% in the fifth or a subsequent decade. The most frequent localization site was cervical region (56.3%). The most frequent symptom was pain-free lymphadenopathy (81.3%). On a macroscopic level, 68.8% of them were found to have lymphatic nodes that were larger than normal. With respect to the histological sub-types, nearly every case was classical HL, 40.6% of the cases were identified as having the mixed cellular type, 40.6% had the nodular sclerosing type and 9.4% had the lymphocyte rich type. Hodgkin/Reed-Sternberg cells were CD15-positive in 91.6% of the cases, CD30-positive in 100% and CD20-positive in 20%.*

***Conclusion**: Our epidemiological data are generally compatible with data from both underdeveloped and developing countries. The results of immunohistochemical staining that we have obtained in this study are in line with the literature data.*

***Key words**: Hodgkin's lymphoma, histopathology, immunohistochemistry.*

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Introduction

Hodgkin's lymphoma (HL), which was originally known as Hodgkin's disease, accounts for nearly 10% of all lymphomas and 0.6% of all cancers. It was identified in 1832, by Thomas Hodgkin. Its etiology, pathogenesis and relation to other types of lymphoma are not known. However, it is believed that the Epstein-Barr virus plays a role in its pathogenesis. HL is a B-cell lymphoma that is characterized by the presence of Hodgkin (lacking nuclear lobation)/Reed-Sternberg (RS) (polylobed) cells and/or their variants[1-6]. Although its pathogenesis is unclear, we aimed to examine the clinical, histopathological and immunohistochemical characteristics of HL cases from a single center in Southeastern Turkey and to discuss them in the light of literature information.

Methods and methods

This retrospective cohort study was performed in the Department of Pathology Unit, Faculty of Medicine, Harran University, Sanliurfa, Turkey. The local ethical committee approved the study. Thirty-two cases that had been diagnosed as HL and of which complete clinical and histopathological information could be fully accessed at our hospital clinics between 2002 and 2013 were included in the present study. Demographic data, presenting

symptoms, and clinical characteristics were obtained from hospital records. All patients had pathologically confirmed HL, had not undergone previous treatment and had no history of other malignancies. The tissue specimens were fixed in 10% neutral buffered formalin solution, processed, and stained with hematoxylin and eosin. Immunohistochemical staining was performed manually. Histologic subtypes were classified according to the World Health Organization criteria as follows: classical HL (nodular sclerosis, lymphocyte rich, mixed cellularity, lymphocyte depleted) and nodular lymphocyte predominant HL (NLPHL). Pathology reports and diagnostic biopsy samples, including sections stained using immunohistochemistry, were retrieved where possible. The clinical, histopathological and immunohistochemical findings were examined retrospectively.

All statistical analyses were performed using SPSS for Windows version 17.0 (SPSS, Chicago, IL, USA). Descriptive statistics were performed and the data were expressed as mean ± standard deviations, minimum, maximum, and percentiles

Results

Of the 32 cases, 22 (68.8%) were male and 10 (31.2%) were female; the male/female ratio was 2.2. The average age of the subjects was 29.9±24.4 (their ages were from 3-87). The distribution of the ages of these cases, in terms of decades, was 31.3% in the first decade, 9.4% in the second decade, 21.9% in the third decade, 6.3% in the fourth decade and 28.1% in the fifth or a subsequent decade. The localization sites of the cases included 56.3% cervical, 12.5% submandibular, 12.5% axillary, 6.2% mediastinal, 6.2% inguinal, 3.2% supraclavicular and 3.1% abdominal. In the clinic, 81.3% of the cases had pain-free lymphadenopathy, 15.6% had fever and pain-free lymphadenopathy and 3.1% had shortness of breath. On a macroscopic level, the diameter of the lymphatic node was under 2 cm in 31.2% of the cases, between 2-4 cm in 46.8% of the cases and more than 4 cm in 22% of the cases. With respect to the histological sub-types, 40.6% of the cases were identified as having the mixed cellular type (MCT) (figure 1), 40.6% had the nodular sclerosing type (NST) (figure 2) and 9.4% had the lymphocyte-rich type (LRT) (figure 3). In 9.4% of the cases, the histological sub-typing could not be performed because of partially fragmented, small and necrotic biopsy material and for various technical reasons. As part of the immunohistochemical study that was conducted, 34.4% of the cases received the CD3 immunostaining, and negative staining was detected in the RS cells of all of these cases. Of the 46.9% of the cases that received CD20 immunostaining, positive staining was detected in the RS cells of 20% of them, and of the 75% of the cases that received CD15 immunostaining, positive staining was detected in the RS cells of 91.6%of them (figure 4).

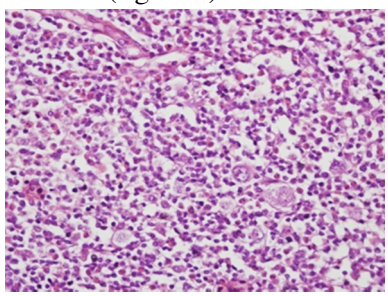

Figure 1: Mixed cellularity Hodgkin's Lymphoma. Hodgkin cells are seen admixed with a polymorphic lymphoid infiltrate rich in eosinophils (Hematoxylin & Eosin x 400).

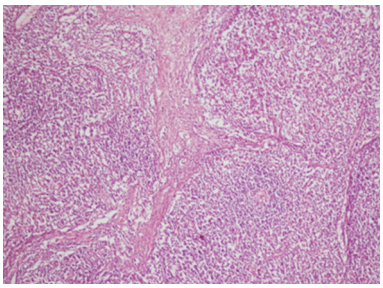

Figure 2: Nodular sclerosing Hodgkin Lymphoma. Broad collagen bands separate the lymphoid tissue into nodules (Hematoxylin & Eosin x 100).

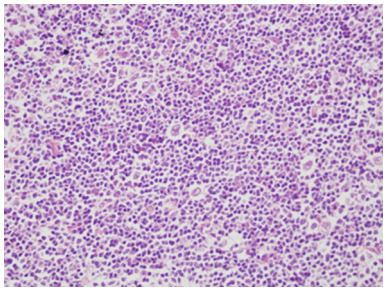

Figure 3: Lymphocyte-rich type Hodgkin Lymphoma (Hematoksilen & Eozin x 400).

In all of the 81.3% of the cases that received CD30 immunostaining, positive staining was detected in the RS cells (figure 5), and among the 37.6% of the cases that received CD45 immunostaining, positive staining was detected in the RS cells of 16.6% of them (Table 1).

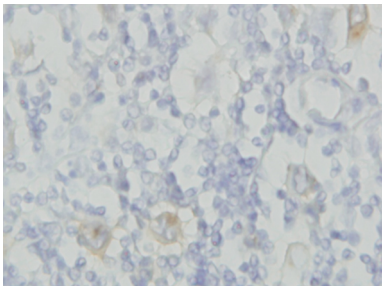

Figure 4: The classic Reed Sternberg cell show positive immunoreactivity for CD15 (CD15 immunohistochemical staining x 1000).

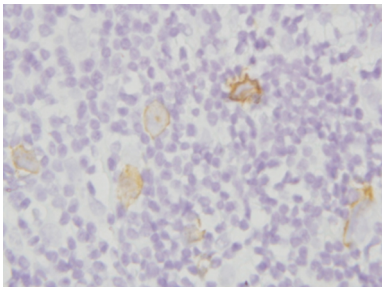

Figure 5: The classic Reed Sternberg cell show positive immunoreactivity for CD30 (CD30 immunohistochemical staining x 1000).

Table 1: Demographical and clinical characteristics of the patients.

Patient characteristics	Patients (N = 32)
Age (years)	29.9±24.4
Gender (M/F)	22/10
Localization, %, (Cervical/submandibular/axillary/mediastinal/inguinal, supraclavicular, abdominal)	56.3/12.5/12.5/6.2/6.2/3.2/3.1
Diameter of the lymphatic node, %, (under 2 cm/between 2-4 cm/over 4 cm)	31.2/46.8/22
Histological subtype, %, (mixed cellular/ nodular sclerosing/lymphocyte-rich/undefined)	40.6/40.6/9.4/9.4
Immunostaining, %, (CD3 and RS cells/CD20 and RS cells/ CD15 and RS cells/ CD30 and RS cells/ CD45 and RS cells)	34.4 and negative/46.9 and 20/75.0 and 91.6/81.3 and 100/37.6 and 16.6

Discussion

It has been noted that the incidence of HL peaks in two decades of life, once in the third decade and again in the fifth decade[7]. While, in industrialized countries it is more frequently found among adolescents and young adults, in the developing countries HL is more common among children[8]. Based on the distribution of our cases in terms of the decades, it was observed that the incidence peaked in the first decade in a similar way to the information from the data obtained from developing countries. HL is also more frequent in men than in women, with the male/female ratio reported as being 1.4[9]. In our cases, the male/female ratio was identified as 2.2, with the males being the dominant group. HL generally emerges with the involvement of lymphatic nodes. It may originate in any lymphatic node site.

However, the cervical and supraclavicular lymphatic nodes are the most frequently involved[1, 2]. Studies by Lukes and Butler have identified that classical HL has most frequently involved the cervical nodes; however, some histological sub-groups have been observed to be more frequent in certain anatomic localizations. These authors found that a great majority of HLs located in the mediastinum were NST, whereas the great majority of HLs located in the abdomen were MCT[10]. In keeping with the literature, the most frequent localization site in our cases was the cervical site; it was also observed that one of the cases with localization in the mediastinum was NST and the other one was LRT. The case with localization in the abdomen was NST. HLs begin in lymph nodes and spread from there to other lymph node groups.

The symptoms of HL are compatible with that of an infectious disease rather than a malignant disease. The main symptom is pain-free lymphadenopathy. Other symptoms that may arise include weakness, fatigue, fever, night-sweating and weight loss, as well as mediastinal lymphadenopathy-related cough, chest pain and hemoptysis[1]. The most frequent symptom among our cases was pain-free lymphadenopathy, which occurred in 81.3% of the cases. In addition, 15.6% of the cases had lymphadenopathy accompanied by fever, and 3.1% experienced shortness of breath.

The dimensions of non-neoplastic lymphatic nodes generally vary between 2 and 20 mm. The cross-section surface is pink in color, looks homogeneous and has a soft texture. In various pathological conditions, the lymphatic node grows and its cross-section surface characteristics change. The lymphatic nodes in HLs are macroscopically larger than normal and have a rubber-like hardness; their cross-section surfaces look like fish flesh. With NST, a prominent nodularity and dense fibrotic bands are observed[1, 2]. In the macroscopic examinations of our cases, 68.8% of them were found to have lymphatic nodes that were larger than normal. It was also observed in the microscopic examinations that the lymphatic nodes consist of a heteroge-

neous cell population that included neoplastic and non-neoplastic reactive cells. Examination also revealed large, mononuclear Hodgkin cells and/or multi-nuclear RS cells or their variants, in varying numbers, scattered on a non-neoplastic inflammatory background. Such tumor cells are generally surrounded by T-lymphocytes in a rosette-like fashion. In addition, the presence of neoplastic and non-neoplastic reactive cells at varying ratios brings about a great number of histological sub-groups. According to the classification made by the World Health Organization in 2008, HLs are divided into two sub-groups: classical HL and NLPHL. Classical HL is divided into four sub-types: NST, LRT, MST and lymphocyte-depletion type (LDT). These subgroups display differences in terms of their morphological, immunophenotypical, genetic and biological characteristics[1, 2, 11]. NLPHL, which is currently accepted as a separate entity, is a monoclonal B cell tumor composed mainly of B lymphocytes and/or histiocytic cells and characterized by a nodular and diffuse pattern[12].

However, it does not include classical HRS cells. Lymphocytic and histiocytic (L&H) cells, called popcorn cells as well, are also generally observed; these are large cells that have a polypoid or multilobe shape with a large, single nucleus and wide, faded cytoplasm. None of our cases had NLPHL. This may be because the incidence of NLPHL among HL cases is just 5% and it is also a new entity that was only defined in 2008. Among classical HLs, the most frequently observed subtype is NST, which accounts for nearly 70% of all cases in developed countries[1]. NST is found less frequently in underdeveloped countries. It is characterized by the collagen bands that surround the lymphoid tissues, separating them into nodules, and also by lacunar cells, a variant of the HRS cell. NST is found at equal frequencies in males and females. The majority of the cases are in the adolescent or young adult age group[3, 13].

In our study, NST made up 40.6% of the cases, which is similar to the data from underdeveloped countries. The average age was 32.2 and most of the cases were males. MCT constitutes nearly 25% of the cases in developed countries, but this percentage is higher in developing countries. It is characterized by a large number of HRS cells within the inflammatory infiltration; these cells are rich in eosinophil leukocyte and composed of plasma cells and lymphocytes. MCT is the most prevalent subtype among male cases and cases where patients are over the age of 50[13, 14]. MCT appeared in 40.6% of our cases and was one of the two most prevalent sub-types, which is similar to the data from developing countries. The average age was 35.2 in our cases and male cases dominated. LRT accounts for 5-10% of all cases, and LDT for less than 5%. LRT is characterized by HRS cells that are located on an inflammatory background that is entirely composed of small lymphocytes. Only 9.4% of our cases were the LRT sub-type, and we did not have any cases of the LDT sub-type. This might be because the incidence of the LDT sub-type is 5% less than the other types and, in addition, recognition the LDT subtypes might have been prevented because of their tendency to become more necrotized than the other HL sub-types[2, 8, 14].

The HLs have a rather large differential diagnosis spectrum that varies according to their subtypes. Diagnosis of HL should be differentiated from the viral lymphadenopathies such as infectious mononucleosis, reactive and atypical lymphoid hyperplasia and reactive lymphadenopathies such as progressive transformation of germinal centers, as well as various lymphomas such as T cell/histiocyte-rich B cell lymphoma, follicular lymphoma, anaplastic large cell lymphoma and small lymphocytic lymphoma. Since there are effective methods for treating this neoplasm, which is fatal if left untreated, it is very important to have its diagnosis differentiated from reactive lymphadenopathies and other types of non-Hodgkin lymphomas[1, 2, 11]. The presence of neoplastic cells such as HRS or L&Hs in the histopathological examination and the presence of their typical immunohistochemical staining patterns are great aids in diagnosis. HRS cells, which are the neoplastic cells of classical HL, are immunohistochemically CD30-positive in nearly 89-100% of cases and CD15-positive in 85-90% of cases and EMA positive in 5-10% of cases. PAX5 and vimentin are generally positive in most HRS cells. Even though CD20 has been reported to be positive in nearly 40% of cases, it is generally negative. HRS cells are also CD79a- and CD45-negative[1, 15].

The immunohistochemical characteristics of L&H cells, the neoplastic cell in NLPHL, are different from those of HRS cells. L&H cells are positive in terms of B-cell antigens such as CD19, CD20, CD22, PAX5 and CD79a, and they are CD45-positive in almost all cases. They commonly express EMA. Almost all of these cases are CD15-negative and CD30-negative; there may be rare

instances of CD30-positivity[1, 16-19]. Nearly every case or ours was classical HL; HRS cells were CD15-positive in 91.6% of the cases, CD30-positive in 100% and CD20-positive in 20%.

After HL cases are diagnosed, the patients are subjected to clinical staging for treatment planning and prognosis identification. The staging is done according to the number of involved lymphatic node sites, their anatomic distribution and the involvement of extra-lymphatic organs or tissues (Ann Arbor Classification). The anatomic spread and histopathological sub-type of the disease are important factors in prognosis identification and optimal treatment selection. In the treatment of HL, the most effective methods are chemotherapy and radiotherapy. These can be applied as monotherapy or combination therapy. Because of the long-term complications of radiotherapy, systemic chemotherapy is generally the preferred option. It is reported that HL cases generally have a good prognosis[13, 20].

In conclusion, this study has shown that our epidemiological data are generally compatible with data from both underdeveloped and developing countries. The results of immunohistochemical staining that we have obtained in our study are in line with the literature data. However, some factors restricted the assessments in our study: it is a retrospective study and we had only a small number of cases. Although histopathological examination is very important, immunohistochemistry is mandatory for diagnosis of HL. We believe that the knowledge and experience we have gained about HLs would be enhanced by having larger series with more cases and studies that include a larger immunohistochemical panel.

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Corresponding Author
Assist. Prof. SEZEN KOCARSLAN
Harran University, Faculty of Medicine
Department of Pathology
Yenisehir campus
63300, Sanliurfa
(Turkey)