Hodgkin's lymphoma

An immunohistochemical profile in northern Iraq

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ABSTRACT

الأهداف: كان الهدف من هذه الدراسة هو تقييم علامات الكيمياء هيستولوجي المناعي في لمفومة هودجكين (HL)، لتحديد نسبة التردد من الأنواع الفرعيةHL .

الطريقة: أدرجت في هذه الدراسة 42 حالة HL خلال الفترة من يناير 2012م إلى يناير 2013م. جمعت هذه الحالات من مستشفى الجمهوري التعليمي ومن المختبرات الخاصة في محافظة نينوى، العراق. خضعت الأورام الفرعية لتصنيف منظمة الصحة العالمية . قيمت علامات (CD30، CD15، CD20، CD79a، CD3، CD43) باستخدام الكيمياء هيستولوجي المناعي.

النتائج: تراوحت أعمار المرضى من 5إلى 81 سنة (32). وقد وضح التوزيع المعري الثنائي، ومع معظم الحالات في العقد الثالث (26.2%). والذكور بالنسبة للإناث 1.6:1. كشف التصنيف الفرعي للأنسجة أن 33 حالة كانت هودجكين متصلب عقيدي(78.6%)، 8 حالات كانت هودجكين مختلطة خلوية (19%).وحالة واحدة كانت غلبة اللمفاوي العقيدي.(2.4%). ظهرت علامات CD30 في جميع حالات لمفومة هودجكين الرئيسية (100%)، كشفت CD15 51.8% من حالاتها. كانت الغلبة اللمفاوي العقيدي CD30 و CD15كانت سلبية، ولكن CD20 ومستضد غشاء الطلائي (EMA) كانت إيجابية. لم يكن هناك علاقة بين CD15 وعمر المرضى وأنواع لمفومة هودجكين الفرعية.

الخاتمة: ظهرت علامات CD30 في (100%)لجميع حالات لمفومة هودجكين الرئيسية، في حينCD15 كانت فقط إيجابية في 51.8%. كان هودجكين المتصلب العقيدي أكثر الأنواع الفرعية شيوعاً في لمفومة هودجكين حيث يمثل 78.6% وتليها هودجكين المختلطة.

Objectives: To evaluate the expression of several immunohistochemical markers in Hodgkin's lymphoma (HL), and to determine the relative frequency of HL subtypes.

Methods: From January 2010 through January 2013, 42 HL cases were included in this case series study. Cases were collected from Al-Jumhori Teaching Hospital and private laboratories in Nineveh province, Iraq. The tumors were subtyped according to the World Health Organization classification system. Several markers including CD30, CD15, CD20, CD79a, CD3, and CD43 were evaluated immunohistochemically.

Results: The patients' ages ranged from 5-81 years (mean: 32). A bimodal age distribution was discerned, with most cases in the third decade (26.2%). Male to female ratio was 1.6:1. Histopathological subtyping revealed that 33 cases (78.6%) were nodular sclerosis HL, 8 cases (19%) were mixed cellularity HL, and one case (2.4%) was nodular lymphocyte-predominant HL. The CD30 marker was expressed in all classical HL cases (100%); while, CD15 was detected in 51.8% of cases. The nodular lymphocyte-predominant HL was CD30 and CD15 negative, but CD20 and epithelial membrane antigen (EMA) were positive. There was no significant relation between CD15 expression, and both patients' age and HL subtypes.

Conclusion: The CD30 marker was expressed in 100% of cases of classical HL, while CD15 was positive in 51.8% only. Nodular sclerosis HL was the most common subtype of HL (78.6%), followed by mixed cellularity HL.

Saudi Med J 2014; Vol. 35 (5): 448-453

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Received 18th September 2013. Accepted 18th March 2014.

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Hodgkin's lymphoma (HL) is defined by the World Health Organization (WHO) as a type of lymphoma in which Reed-Sternberg cells (RS cells) are present in a characteristic reactive inflammatory background.[1,2] The age of onset of HL shows a bimodal distribution with a first peak at young adulthood and a second peak after the age of 60 years.[3,4] Hodgkin's lymphoma was divided according to Rye's classification in 1966 into 4 classes.[5] This classification was adopted from 1966 until 1999 when a revised European-American classification of lymphoma (REAL) was proposed and adopted by the World Health Organization (WHO).[5] This REAL/WHO system reclassified HL into:[2] classical HL (CHL), which included the Rye's 4 subclasses (lymphocyte predominant [nodular or diffused], nodular sclerosis [NSHL], mixed cellularity [MCHL], and lymphocyte depleted), and nodular lymphocyte-predominant HL (NLPHL). Hodgkin's Reed Sternberg (HRS) cells are typically positive for CD15 and CD30, and often lack expression of pan B-cell markers (CD19, CD20, CD22, CD45, and CD79a).[6-21] The HRS-cells, also express the Pax-5/B-cell-specific activator protein.[12,13] The lymphocyte predominant (LP) cells of NLPHL are CD45+, express the B-cell associated antigens CD19, CD20, CD22, CD79a, but are negative for CD30 and CD15, contrary to the pattern from true HRS cells.[7,8] A subset of LP cells (approximately 40%) express epithelial membrane antigen (EMA), whereas true HRS cells are negative.[8] The CD3 is a marker for T cells and natural killer cells.[22] It is specific for T-cell derivation, and is found in a minority of HL cases.[23] The CD43 or leukosialin is expressed on leukocytes and has been shown to be involved in T-cell proliferation.[24] In addition to the diagnostic role played by immune markers for HL, immunohistochemistry also has an important role in the selection of appropriate treatment.[4,10,11] The current study intends to evaluate the expression of several immunohistochemical markers namely (CD15, CD30, CD20, CD79a, CD3, and CD43) in HL, to determine the relative frequency of HL subtypes, and to compare the results with those of others.

Methods. The Research Ethics Committee, in the College of Medicine, University of Mosul, Mosul, Iraq approved this work. In this case series study, extending over a 3-year period from January 2010 through January 2013, all cases diagnosed as HL at Al-Jumhori Teaching Hospital and those referred from private laboratories in Nineveh province in the North of Iraq were enrolled in the current study. This study includes 42 cases of HL; all histopathological reports were reviewed regarding clinicopathological data (age and gender). For each case, the authors reviewed hematoxylin and eosin stained sections. The tumors were diagnosed and subtyped according to the WHO classification system, 2008.[2] A panel of several immune markers, including CD 30, CD15, B-cell markers (CD20 and CD79a) and T-cell markers (CD3 and CD43), were assessed immunohistochemically on formalin-fixed paraffin-embedded (FFPE) tissues of the tumor, using mouse monoclonal antibody, and the LSAB2 detection system with DAB chromogen (Dako company, Carpitera, CA, USA). Positive and negative control slides were involved in each run of staining. For the evaluation of marker expression, histopathological features of HRS cells are observed in addition to chromogen staining pattern in such cells, which are compared with the control slides. For CD30 and CD15, both membranous and paranuclear Golgi apparatus staining are regarded positive, whereas membranous staining is considered positive for the other markers including CD20, CD79a, and CD43, and cytoplasmic staining is considered positive for CD3.

All immunohistochemical markers and clinicopathological variables were analyzed using the Statistical Package for Social Sciences version 19 SPSS Inc., Chicago, IL, USA). A probability of 95% was used to assign significant end results.[25]

Results. The patients ranged in age from 5 to 81 years (mean: 32). There is a bimodal age distribution of cases (59.5% in the first 3 decades and 28.5% in the fifth and sixth decades), with a peak incidence in the third decade (26.2%) (**Figure 1**). Also, predominance of male gender is noticed with a male to female ratio of 1.6:1. Histopathological sub typing revealed that 33 cases (78.6%) were NSHL (**Figure 2**), 8 cases (19%) were MCHL, and one case (2.4%) was NLPHL. The CD15 detection was applied in 28 cases, of them one case was NLPHL, and 27 cases were CHL (22 NSHL, and 5 MCHL), 51.8% cases were positive (n=14) as shown in **Figure 3**, whereas 48.2% were negative (n=13). Among NSHL, 11 cases were CD15 positive (50%), and 11 were CD15 negative (50%), while 60% of MCHL were CD15 positive (3 cases out of 5). There was no relation between CD15 expression and both patients' ages (p=0.352), and HL subtypes (p=0.686).

Disclosure. Authors have no conflict of interests and the work was not supported or funded by any drug company.

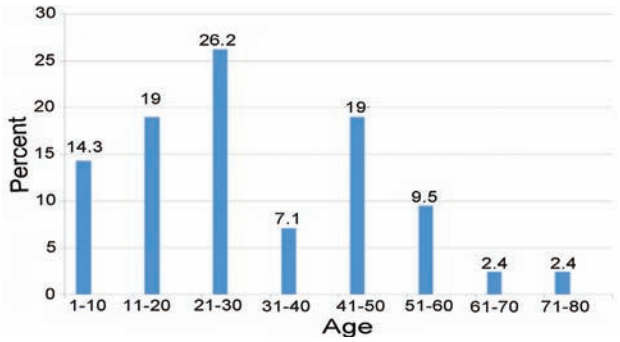

Figure 1 - Age distribution of Hodgkin's lymphoma patients showing bimodal pattern.

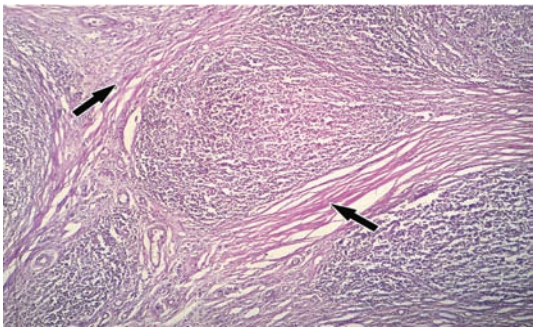

Figure 2 - Nodular sclerosis Hodgkin's lymphoma, showing fibrous bands surrounding nodular aggregates of lymphoid cells (arrows) (Hematoxylin & Eosin x100).

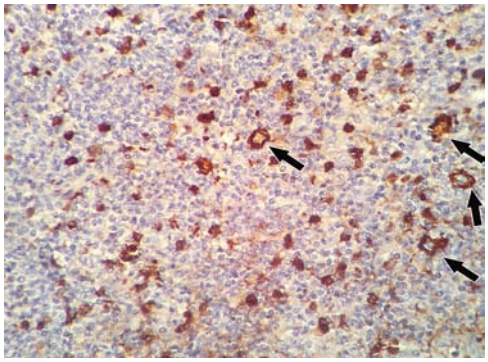

Figure 3 - Mixed cellularity Hodgkin's lymphoma, showing membranous and paranuclear dot-like CD15 positive immunostaining (arrows) (x400).

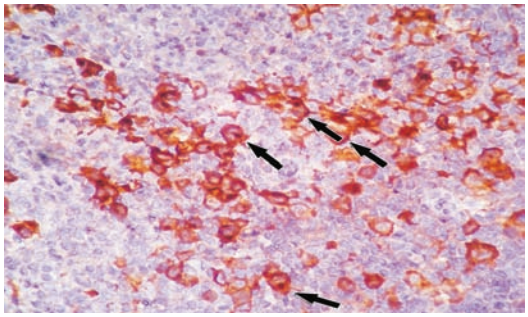

Figure 4 - Nodular sclerosis Hodgkin's lymphoma, showing membranous and paranuclear dot-like CD30 positive immunostaining (arrows) (x400).

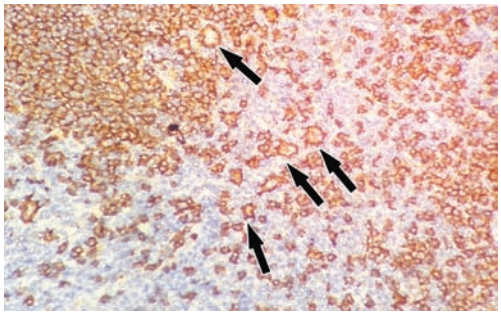

Figure 5 - Nodular lymphocyte predominant Hodgkin's lymphoma, showing membranous CD20 positive lymphocyte predominant cells (arrows) with CD20 positive lymphocytes in the background (x400).

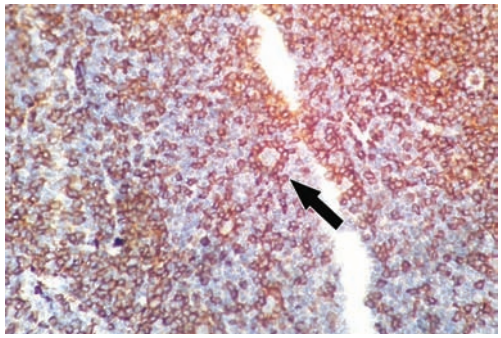

Figure 6 - Nodular lymphocyte predominant Hodgkin's lymphoma, showing cytoplasmic CD3 positive immunostained T-lymphocytes forming rosettes around lymphocyte predominant cells (arrow) (x400).

The CD30 marker expression was observed in all cases of CHL (n=41), (**Figure 4**). The cellular background of HL was a mixture of lymphocytes, histiocytes, and granulocytes in addition to eosinophils. Most of the lymphocytes were of T-cell types and showed reactivity for CD3, and CD43. However, some reactive B-cells were observed to be positive for CD20 and CD79a. The RS cells of classical HL were negative for CD3, CD43, CD20, and CD79a. Only one case of NLPHL was diagnosed, in which the LP cells were negative for both CD 30 and CD15. However, positive staining was detected for both EMA and CD20 (**Figure 5**). The surrounding reactive cells showed reactivity for CD3 in a rosette-like pattern (**Figure 6**).

Discussion. Hodgkin's lymphoma is a lymphoid tumor that accounts for less than 1% of all de novo neoplasm's occurring every year worldwide.[1,5] Despite

its well known histological and clinical features, HL has recently been the subject of intense research activity, in order to have a better understanding of its phenotype, molecular characteristics, histogenesis, and possible mechanisms of lymphogenesis.[1,5,6]

In economically developed countries of the East and West, HL has a characteristic bimodal age distribution with a peak in the second and third decades, and another peak after the seventh decade of life.[26-28] This is similar to the current study findings, though the second peak was in younger age groups (fifth and sixth decades). This may be attributed to a long life expectancy and early diagnosis and management of patients with HL in Western countries. In the current study, the patients' distribution among age groups also confirms the previous figures of the Iraqi/Mosul cancer registries,[29,30] and is in concordance with other studies from Kuwait,[31] Iran,[32] India,[33] and Malaysia.[34] Several other studies from Bahrain,[35] Saudi Arabia,[36] and Pakistan[37] observed dominance of HL cases in children and adolescents, but not a second peak in the elderly.

The incidence of HL in Asian women is lower than that of men, but the overall pattern is similar.[37,38] This is obvious in this study as males formed most cases (male to female ratio was 1.6:1). This is comparatively less than reports from Duhok in Northern Iraq (M:F=3:1),[39] but is closer to the ratios reported from Turkey (1.56:1),[40] Jordan (1.5:1),[41] Saudi Arabia (1.4:1),[42] and European countries.[43,44]

In the current study, NSHL formed the most frequent histological subtype, which contrasts with earlier reports from Iraq,[45,46] and nearby countries,[47,48] as well as Egypt,[49] in which the mixed cellularity subtype was the most frequent. This changing pattern is consistent with a more recent local study[50] and also reports from Saudi Arabia,[51] UAE,[52] and Kuwait,[53] where higher relative rates of NSHL were reported approaching those found in the United States[26] and Europe.[54] Al-Diabe et al[36] from Saudi Arabia construed that this change may be due to urbanization and improvement in living standards. This might have led to a reduced risk of early childhood exposure to Epstein Barr virus that is more likely to be associated with MCHL rather than NSHL.[36]

The immunohistochemical assessment of CD markers is an important parameter in the evaluation and classification of HL. Although the CD marker status provides prognostic information, currently its major clinical value lies in the identification of these markers in HL subtypes, which has led to a rationale for many observations concerning the responses of advanced and recurrent HL subtypes to chemotherapy. In the current study, CD15 was expressed in 51.8% of CHL cases. This result is comparable with those reported from India,[55] and the Ukraine,[56] who found CD15 positivity in approximately in 55.5% (India), and 58.2% in (Ukraine) When compared with a study from China,[57] the current study showed higher rates of CD15 expression, while it is lower than reports from Egypt,[58] Turkey,[59] Europe,[60-62] and North America.[63] The reasons for this wide range of detection rates may be attributed to the properties of different antibodies, the tissue fixation procedure, protocols, variation in the technique of incubation and antigen retrieval, and subjectivity in interpretation, as well as the number of the cases studied. Also differences in population groups, diversity of risk habits, and variation of genetic predisposition may also have contributing roles.

We found no statistical significance between CD15 expression and both patients' ages and HL subtypes, perhaps due to the small sample size studied. All of the reported cases of CHL in the current study show CD30 expression, and a similar finding was noticed in previous studies.[57,58,60,62] Most of the studies, similar to this work, noticed that CD30 is expressed in HRS cells in a higher proportion compared with CD15. As CD15 and CD30 are also expressed in other tumors including those of hematolymphoid origin, the combination of them in a panel is more useful in diagnosing CHL.

The NLPHL is a unique subtype of HL with characteristic morphologic, biologic, and clinical features. In the current study, only one case was reported (2.4%). This is similar to the observations of Yaqo et al in Northern Iraq (4.8%),[39] and also concordant with the infrequently described cases in similar work in Baghdad (4 cases over a 2 year period).[5] Immunohistochemistry is a valuable mean in the detection of NLPHL cases, because many are included in the classical category of HL when using H&E stained sections alone. The LP cells were negative for CD15 and CD30, but reactive to CD20 and EMA, surrounded by easily identifiable CD3+ T-cell rosettes. Such an immunophenotype is classical of NLPHL, and comparable with the findings of many other local, regional, and global studies.[5,64,65]

The diverse cellular infiltrate of lymph nodes in HL, with T- lymphocytes predominating over B-cells, has been noticed in the current work as well as others.[66,67] This has been attributed to various cytokines mediating reciprocal cross talk between HRS cells and the surrounding cellular milieu.[67] The cytokines produced by T cells may help the growth and/or survival of HRS cells.[67] The production and induction of various other cytokines may also explain the influx of eosinophils (IL-5, and eotaxin) and plasma cells (IL-6).[67] Differences in chemokine and cytokine production may also be responsible for the differences between the histological subtypes of HL.[67]

The limitation of this study is the current circumstances of our country, which have an effect on sample size and adequate follow up of patients. As a result, a relationship between the immune profile and patient's survival cannot be obtained.

In conclusion, CD30 was expressed in 100% of cases of CHL, while 51.8% were CD15 positive. There was single case of NLPHL, which was negative for both CD15 and CD30, but positively stained for CD20 and EMA. The NSHL was the most common subtype of HL in this locality accounting for (78.6%) followed by MCHL, which represented (19%). Hodgkin's lymphoma has a bimodal age distribution with predominance of male patients; however, the second peak was at a younger age. The peak age of incidence of HL was in the third decade.

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