Journal of Enam Medical College
Vol 5 No 1 January 2015

Original Article

Significance of Immunohistochemistry in Accurate Characterisation of Hodgkin Lymphoma

Mahtab Uddin Ahmed[1], Harun-Or-Rashid Khan Shilpi[2]

Received: September 3, 2014 Accepted: November 23, 2014 doi: 10.3329/jemc.v5i1.21493

Abstract

***Background**: Hodgkin lymphomas are malignant disorders of cells residing in lymphoid tissue and containing Reed-Sternberg (RS) cells and its variants and account for about 0.7% of all new cancers. Hodgkin lymphomas are two types: Classical Hodgkin lymphoma (CHL) that is CD30 positive and CD45 negative in specific pattern; Lymphocyte-predominant Hodgkin lymphoma (LPHL) that is CD30 negative and CD45 positive in specific pattern. **Objective**: To evaluate the value of immunohistochemistry in the diagnosis and accurate characterisation of Hodgkin lymphoma. **Materials and Methods**: This cross-sectional study was carried out in the department of Pathology of Sir Salimullah Medical College & Mitford Hospital, Dhaka from January 2010 to June 2012. Histopathologically diagnosed 45 cases of Hodgkin lymphoma and 5 cases of other than Hodgkin lymphoma were selected and then immunomarkers CD30 and CD45 were applied. **Results**: Among 50 cases 37 were classical Hodgkin lymphoma and 8 cases were lymphocyte-predominant Hodgkin lymphoma histopathologically. When immunomarkers were applied in 50 cases then 33 cases were classical Hodgkin lymphoma and 9 cases were lymphocyte-predominant Hodgkin lymphoma. **Conclusion**: Immunohistochemistry helped in accurate characterisation of Hodgkin lymphoma.*

***Key words**: Hodgkin lymphoma; Histopathology; Immunohistochemistry*

J Enam Med Col 2015; 5(1): 23–29

Introduction

Lymphomas are malignant disorders of cells residing in lymphoid tissue and are classified into two main types: Hodgkin lymphoma and non-Hodgkin lymphoma. About 1,700 cases are diagnosed as Hodgkin lymphoma in United Kingdom each year. About 1 in every 200 cancers diagnosed is a Hodgkin lymphoma.[1] Hodgkin lymphomas account for 0.7% of all new cancers in the United States. Male female ratio in Hodgkin lymphoma is 1.5:1 (except nodular sclerosis).[2]

Hodgkin lymphomas are of two types: a) Classical Hodgkin lymphoma (CHL), b) Lymphocyte-predominant Hodgkin lymphoma (LPHL). These two entities of Hodgkin lymphoma differ in their clinical features and behaviour, and in their morphology, immunophenotype and immunoglobulin transcription of the neoplastic cells as well as in the composition of their cells and their cellular background.[3] LPHL have distinct pathobiology: an indolent natural history, unimodal age distribution, marked male predominance, frequent axillary presentation, and association with low stage disease. Long survival of patient with LPHL without any treatment could favour a 'watch and wait' strategy.[4]

There are some other conditions in which Reed-Sternberg (RS)-like cells are seen — these include infectious mononucleosis, non-Hodgkin lymphoma, graft-versus-host disease, tuberculous lymphadenitis, cat-scratch disease, sarcoidosis, human immune deficiency virus infection, metastatic cancer, drug induced pseudolymphoma.[5] These disorders enter the differential diagnosis of Hodgkin lymphoma. Most tumours suspected as being lymphocyte depletion

1. Assistant Professor, Department of Pathology, Bangladesh University of Health Sciences, Dhaka
2. Associate Professor, Department of Pathology, Sir Salimullah Medical College, Dhaka
***Correspondence** Mahtab Uddin Ahmed, Email:mahtab.path@yahoo.com*

Hodgkin lymphoma are actually proved to be large cell non-Hodgkin lymphoma. In 3–5% cases of lymphocyte-predominant type Hodgkin lymphoma resembling diffuse large B cell lymphoma, marker study can help to resolve the problem.[3]

The marker CD30 is positive and marker CD45 is negative in RS cells in classical Hodgkin lymphoma. On the other hand study with the same markers on RS variant L&H cells (popcorn cell) present in nonclassical lymphocyte-predominant variety resulted in opposite finding, that is, the cell is negative for CD30 but positive for CD45 in specific pattern.[5]

Immunohistochemistry (IHC) is a new technique in our country. As IHC has provided a feasible approach to performing immunostaining on routinely processed tissues, and this method is now becoming a routine practice in surgical pathology laboratories in some hospitals, we designed to conduct this study in our population for accurate diagnosis and characterisation of Hodgkin lymphoma using the two immunohistochemical markers CD30 and CD45.

Materials and Methods

This cross-sectional study was carried out in the department of Pathology of Sir Salimullah Medical College & Mitford Hospital, Dhaka from January 2010 to June 2012. Forty five histopathologically diagnosed Hodgkin lymphoma biopsy specimens and 5 cases other than Hodgkin lymphoma, 3 non-Hodgkin lymphoma and 2 reactive changes, of both sexes were purposively included in this study. Among the Hodgkin lymphoma cases 37 were classical Hodgkin lymphoma and 8 were lymphocyte-predominant Hodgkin lymphoma. The patients were admitted in Mitford Hospital, Dhaka Medical College Hospital, BSMMU, Delta Hospital, LABAID Hospital, SQUARE Hospitals and different nearby clinics with lymphadenopathy for the purpose of management. Immediately after operation the specimens (lymph nodes) were collected and blocks were prepared for both hematoxylin and eosin stain and for immunohistochemistry. After diagnosis by histopathology in hematoxylin and eosin stain, the remaining blocks were used for immunohistochemistry. Considering histopathology as the gold standard[6], the results of immunochemistry were compared. Biopsy specimens with poorly fixed sections, sections with extensive tissue or cell necrosis, specimens sent in the form of aspirates were excluded.

Study procedure

In this study comparison between two diagnostic procedures, histopathology and immunochemistry, were done for characterisation of Hodgkin lymphoma. After selection of specimens immunomarkers CD30 and CD45 were applied on each of these. By seeing specific staining pattern of immunomarkers, characterisation of Hodgkin lymphoma were done and compared with histopathology using diagnostic performance tests (sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV] and accuracy).

Immunohistochemical staining procedure

Immunohistochemistry was done on the tissue specimens histopathologically diagnosed as Hodgkin lymphoma. Paraffin embedded specimens were sectioned at a thickness of 4–5 microns. Sections were deparaffinised and rehydrated through graded alcohol to water prior to staining proper. Antibodies for CD30 and CD45 were used in all cases for diagnosing and characterisation of subtypes of Hodgkin lymphoma.

Routine staining for light microscopy

Tissues were fixed in 10% formalin and embedded in paraffin block and processed routinely. The paraffin blocks were sectioned at 4–5 micrometer thickness and were stained with H&E stain.

Ethical clearance

Prior to the commencement of the study, the research protocol was approved by the Ethical Committee of Sir Salimuallah Medical College & Mitford Hospital, Dhaka. There was no threat or risk for study population. Patients' private information was not disclosed.

Data processing and analysis

All the data were checked and edited after collection and computerised. Statistical analysis of the results was obtained by using Windows based computer software devised with Statistical Packages for Social Sciences (SPSS 13.0) (SPSS Inc, Chicago, IL, USA). The results were presented in tables and figures. The statistical terms included in this study are mean, standard deviation and percentage. Statistical significance was set at $p<0.05$ and confidence interval set at 95% level.

Results

The age of the subjects in this study was 7–79 years with mean ± SD 33.90 ± 17.70 years. Table I shows the

sex distribution of Hodgkin lymphoma patients. Male to female ratio was 1.64:1. Table II shows the sites of lymphadenopathy of Hodgkin lymphoma. All these lymphadenopathies were superficial. Histopathlogical types of Hodgkin lymphoma are shown in Table III.

Table I: Sex distribution of Hodgkin lymphoma patients (n=45)

Sex	Frequency	Percentage
Male	28	62.23
Female	17	37.77
Total	45	100.0

Table II: Sites of lymphadenopathy of Hodgkin lymphoma (n=45)

Sites	Number	Percentage
Generalised	4	8.88
Localized		
Axial		
Cervical	28	62.22
Supraclavicular	8	17.77
Peripheral		
Axillary	5	11.11

Table III: Histopathological types of Hodgkin lymphoma (n=45)

Histopathological types	**Number**	**Percentage**
Classical		
Nodular sclerosis	20	44.44
Mixed cellularity	12	26.66
Lymphocyte rich	03	6.66
Lymphocyte poor	02	4.44
Lymphocyte-predominant	08	17.78

Table IV shows that among 45 cases of Hodgkin lymphoma 32 (71.11%) were CD30 positive and CD45 negative in specific pattern and 9 (20%) cases were CD45 positive in specific staining pattern and CD30 negative. Four (8.8%) cases showed no staining pattern. Among five cases which were other than Hodgkin lymphoma, 2 reactive and 2 non-Hodgkin lymphoma showed both CD30 and CD45 negativity in specific pattern of staining; but one non-Hodgkin lymphoma showed CD30 positive-like RS cells and CD45 negativity in specific pattern (Table V).

Table IV: Immunohistochemisty findings of Hodgkin lymphoma subjects (n=45*)

Histopathological types		CD30	CD45
Classical	Nodular sclerosis (n=18)	+ve	–ve
	Mixed cellularity (n=12)	+ve	–ve
	Lymphocyte poor (n=2)	+ve	–ve
	Lymphocyte rich (n=2)	–ve	+ve
Lymphocyte predominant	Lymphocyte predominant (n=7)	–ve	+ve

* Four cases showed no staining pattern

Classical lymphoma: CD30 +ve, CD30 shows specific cell membrane staining and dot like cytoplasmic staining of RS cells in case of nodular sclerosis, mixed cellularity and lymphocyte poor Hodgkin lymphoma (Figures 1 and 2); but lymphocyte rich lymphoma shows no specific staining pattern (CD30 –ve). CD45 –ve, CD45 shows nonspecific diffuse staining pattern in case of nodular sclerosis, mixed cellularity and lymphocyte poor Hodgkin lymphoma; but lymphocyte rich lymphoma shows specific popcorn cell membrane stainning pattern (CD45 +ve). Lymphocyte-predominant Hodgkin lymphoma: CD45 +ve, CD45 shows specific popcorn cell membrane (Figures 3 and 4); CD30 –ve, CD30 shows no specific staining pattern.

Table V: Immunohistochemisty results in five cases other than Hodgkin lymphoma

Histopathological types	CD30	CD45	Description of staining by CD30	Description of staining by CD45
Reactive change (n=2)	–ve	–ve	No specific staining pattern	Nonspecific diffuse staining pattern of lymphocytes
Non-Hodgkin lymphoma (n=2)	–ve	–ve	No specific staining pattern	Nonspecific diffuse staining pattern of lymphocytes
Non-Hodgkin lymphoma (n=1)	+ve	–ve	CD30 shows specific staining pattern-like cell membrane and dot like cytoplasmic staining of RS cells	Nonspecific diffuse staining pattern of lymphocytes

Table VI: Diagnostic performance test results (n=50=45+5)

Immunohistochemistry	Histopathology		Total
	Classical	Lymphocyte predominant	
Classical	32 (TP)	1 (FP)	33
Lymphocyte-predominant	2 (FN)	7 (TN)	9
Total	34	8	42

TP, True positive; FP, False positive; FN, False negative; TN, True negative

Table VII shows the findings of validity test. The sensitivity, specificity, PPV, NPV and accuracy are 94.11%, 87.5%, 96.96%, 77.78% and 92.85% respectively.

Table VII: Diagnostic performance test of immunohistochemistry compared with histpathology as gold standard

Tools	Value (%)
Sensitivity	94.11
Specificity	87.5
PPV	96.96
NPV	77.78
Accuracy	92.85

PPV, Positive predictive value; NPV, Negative predictive value

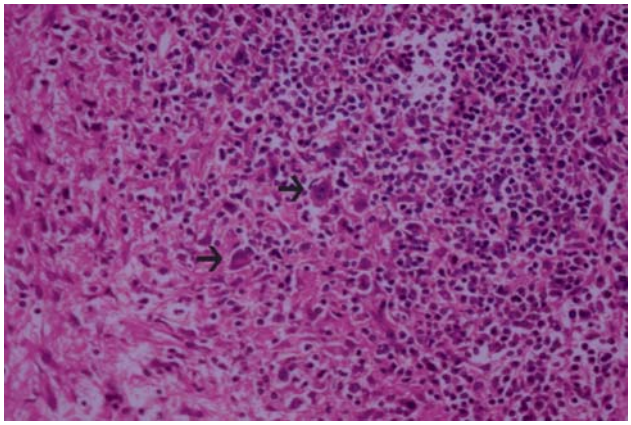

Fig 1. Photomicrograph of classical Hodgkin lymphoma showing CD30 +ve cells (H&E stain)

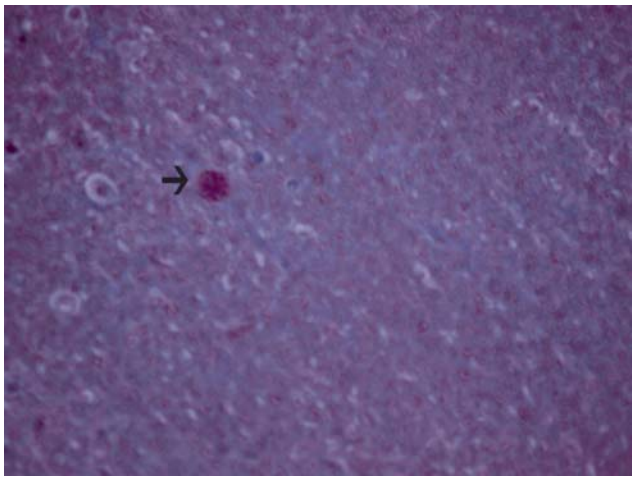

Fig 2. Photomicrograph of classical Hodgkin lymphoma showing CD30 +ve RS cells

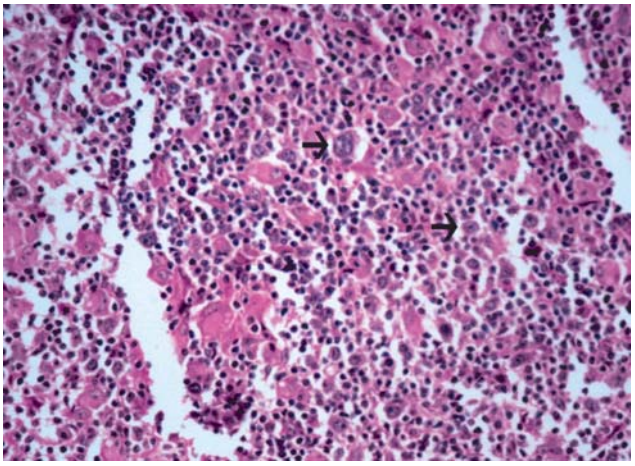

Fig 3. Photomicrograph of lymphocyte-predominant Hodgkin lymphoma showing CD45 +ve cells (H&E stain)

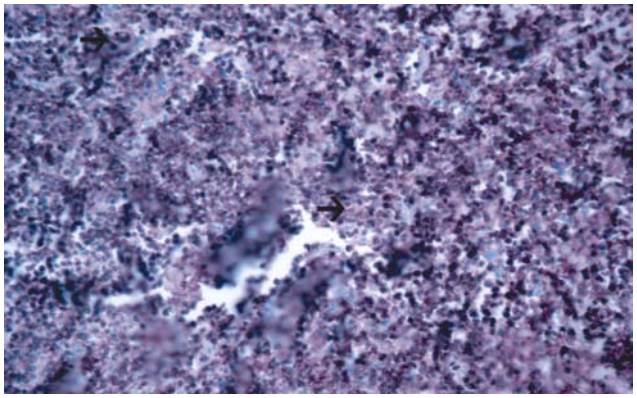

Fig 4. Photomicrograph of lymphocyte-predominant Hodgkin lymphoma showing CD45 +ve L&H (popcorn) cells

Discussion

In this study, among 50 cases 45 were histopathologically diagnosed as Hodgkin lymphoma of which 37 cases were classical Hodgkin lymphoma and 8 cases were lymphocyte-predominant Hodgkin lymphoma. Among the 45 cases of Hodgkin lymphoma 20 (44.44%) were nodular sclerosis variety, 12 (26.66%) were mixed cellurity variety, 3(6.66%) were of lymphocyte rich variety and 2 (4.44%) cases were of lymphocyte poor variety. In a previous study it was shown that nodular sclerosis accounted for 70% and mixed cellularity variety accounted 15–25% of classical Hodgkin lymphoma.[7]

Out of 45 cases of Hodgkin lymphoma, 28 (62.22%) were male patients and 17 (37.77%) were female. Male to female ratio was 1.64:1. Desforges et al[8] stated that in their study younger group showed equal male to female ratio in contrast to higher male to female ratio in older age group in Hodgkin lymphoma. Bhatia[9] found male to female ratio 1.47:1. Rosillo[10] found male to female ratio 1.8:1 in 127 patients with Hodgkin lymphoma.

In this study, among all the 45 cases of Hodgkin lymphoma, lymphadenopathy was superficial, of which 28 (62.22%) were cervical, 8 (17.78%) supraclavicular and 5 (12.19%) axillary. In this study supradiaphragmatic involvement of lymph node was more than subdiaphragmatic level. Kim & Dorfman[11] found mediastinal involvement in 17.0% cases of Hodgkin lymphoma.

Regarding immunohistochemistry study in 45 cases of Hodgkin lymphoma, 32 were CD30 positive and CD45 negative in specific staining pattern and 9 cases were CD45 positive in specific staining pattern and CD30 negative. Out of five cases other than Hodgkin lymphoma, four cases were both CD30 and CD45 negative in specific staining pattern; only one case was CD30 positive and CD45 negative in specific pattern. Lymphocyte-predominant Hodgkin lymmphoma may differentially be diagnosed as lymphocyte rich classical Hodgkin lymphoma.[12] Histopathologically classical RS cells have either bilobed nuclei or two nuclei, each containing distinct nucleoli and L&H cells have a single, large, folded or multilobated nucleus (popcorn cell). L&H cell nuclei typically contain multiple small basophilic nucleoli that are smaller than those of classical RS cells.[3] Curiously, the European Task Force on Lymphoma (ETFL) study reported the coexistence of RS cells in addition to L&H cells in substantial percentage of LPHL cases.[8] The current consensus, however, is that although cells that appear to be RS cells by morphologic criteria can occasionally be seen in LPHL, such cells are negative for CD15 and CD30 and positive for CD45 and therefore are not classical RS cells.[13]

Lymphocyte-predominant Hodgkin lymphoma occurs in 5–10% cases of Hodgkin lymphoma.[13] But number of cases of lymphocyte-predominant Hodgkin lymphoma was higher (17.78%) in this study. Lymphocyte-predominant Hodgkin lymphoma occurs mainly in cervical and axillary regions.[13] In the present study, maximum number of lymphadenopathy occurred in cervical (n=28) and axillary (n=5) regions. Submental, inguinal and mesenteric lymph node swellings were absent.

Lymphocyte-predominant Hodgkin lymphoma occurs mainly in 30–40 years with a male predominance.[13] In this study, overall mean age was 33.90 years and number of males was 28 (62.22%) and that of females was 17 (37.78%).

In this study CD30 stained RS cell membranes and showed dot like cytoplasmic staining in cases of classical Hodgkin lymphoma and no specific staining pattern in cases of lymphocyte-predominant Hodgkin lymphoma. In few cases CD30 stained centroblast and centrocytic cells of lymphoma. Jaffe[14] also got similar staining pattern.

In this study CD45 stained popcorn cell membranes of lymphocyte-predominant Hodgkin lymphoma whereas there was no specific staining pattern in classical Hodgkin lymphoma; but it showed diffuse staining pattern by staining all haemopoetic cells of classical Hodgkin lymphoma. Lee[15] also got the same findings.

In 2 reactive changes and 2 non-Hodgkin lymphoma cases of 5 cases other than Hodgkin lymphoma, CD30 showed no staining and CD45 showed diffuse staining of haemopoietic cells like T cells and B cells. Similar staining patterns were also found by other researchers.[16,17]

In one case of non-Hodgkin lymphoma, CD30 showed staining that was like RS cells. CD45 showed diffuse staining pattern of haemopoietic cells in non-Hodgkin lymphoma. Gutenson & Cole[17] and Hall[18] also found similar findings.

In the present study histopathological diagnosis was taken as the diagnostic gold standard. Compared with this the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of immunohistochemistry were 94.11%, 87.5%, 96.96%, 77.78% and 92.85% respectively. In one previous study using immunomarkers CD45 and CD30 in Hodgkin lymphoma, the sensitivity, specificity, PPV and NPV of haematoxylin and eosin staining were 76.61%, 92.75%, 96.32% and 61.53% respectively.[19]

Among 45 cases of Hodgkin lymphoma, using immunochemistry 32 (71.11%) cases were classical and 9 (20%) cases were lymphocyte-predominant Hodgkin lymphoma. Three cases of classical and one case of lymphocyte-predominant Hodgkin lymphoma showed no staining due to potential problems such as weak target antigen staining, primary/secondary antibody cross reactivity or improper fixation. In case of classical (CD30+, CD45–) cases positive controls and in case of lymphocyte-predominant (CD45+, CD30–) cases negative controls were used in immunohistochemistry.

Immunohistochemistry (IHC) is built on the foundations of histopathology. It does not replace histopathology but rather serves as a valuable adjunct that greatly extends the variety of tissue components that can be demonstrated specifically within tissue section or other cell preparations. The basic critical principle of IHC, as with any other special staining method, is a sharp localisation of target components in the cell and tissue based on a satisfactory signal. Amplifying the signal while reducing non-specific background staining is a major challenge to achieve a satisfactory and practically useful result.[9]

From the findings of the present study it can be concluded that immunohistochemistry has high sensitivity, specificity and diagnostic accuracy in case of Hodgkin lymphoma and it can safely be used in the diagnosis of Hodgkin lymphoma along with histopathology.

Limitations of the study

The major limitation of this study is small sample size; only 50 cases were included in the study. Secondly, there are many markers for Hodgkin lymphoma, but due to difficulty in procurement and high cost, only two markers (CD30 and CD45) were used in this study. There are also many potential problems affecting the outcomes of the immunostaining procedure. There were reasons to believe that the findings and conclusion of the study may not be completely representative though it may give general idea of certain clinicopathological aspect of Hodgkin lymphoma amongst the population of Bangladesh.

References

1. Prevalence and incidence of lymphoma. Available at: http://www.wrongdiagnosis.com/1/lymphoma/prevalence.htm. Accessed December 2010.
2. Abeer MES, Mohammad HEB, Abeer AB, Sadia MEG. Flow cytometric immunophenotyping (FCI) of lymphoma: correlation with histopathology and immunohistochemistry. Available at: http://www.ncbi.nlm.nih.gov/ pmc/articles/ PMC2637251. Accessed April 2010.
3. Ahmed Z, Azad NS, Bhurgari Y, Ahmed R, Kayani N, Pervez S et al. Significance of immunohistochemistry in accurate characterization of malignant tumors. Department of Pathology and Microbiology. The Aga Khan University of Hospital, Karachi, Pakistan. Available at: zubair.Ahmed@ AICU.edu. Accessed December 2010.
4. Alison RE, Rush RS. Natural history of Hodgkin's disease as related to staging. Cancer 1966; 10(19): 308–316.
5. Lymph node. In: Rosai J (ed). Rosai and Ackerman's surgical pathology. 9th edn. St. Louis: Mosby, 2004: 1877–2018.
6. Ayalew T, Robin A, Zallers, Peters M, Banks, Terry M et al. Clinical correlates of distinct immunophenotypic and histologic subcategories of lymphocyte-predominance Hodgkin's disease. Journal of Clinical Oncology 1990; 8(12): 1959–1965.
7. Bashyal R, Pathak TB, Shrestha S, Pun CB, Banstola S, Neupane S et al. Role of immunohistochemistry in the diagnosis of malignant small round cell tumors. Journal of Pathology of Nepal 2011; 1: 87–91.
8. Desforges JF, Ratherford CJ, Piro A. Hodgkins disease. J Med 1979; 30(1): 2112–2122.
9. Bhatia S. Immunohistochemistry – an important adjunct to surgical pathology. Available at: Medical Buyers Website. Accessed June 2010.
10. Rosillo SP. Complication of total nodal irradiation of Hodgkin's disease in stage III and IV. Cancer 1990; 42: 437–447.
11. Kim H, Dorfman RF. Morphological studies of 84 untreatable patients subjected to laparotomy for staging of non-Hodgkin's lymphoma. Cancer 1974; 33: 657–674.
12. Armitago JO, Longo DL. Hodgkin disease. Nodular lymphocyte predominant Hodgkin disease. In: Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL

(eds). Harrison's principles of internal medicine. 15th edn. New York: McGraw-Hill, 2001: 725–730.

13. Drug on Hodgkin's lymphoma. Available at: www.drug.com/enc/Hodgkinlymphoma. Accessed December 2010.
14. Jaffe ES. Pathology and genetics tumour of hematopoetic and lymphoid tissue. Available at: http://www.medinidia.net/patients/patientinfo/ hodgkinlymphoma. Accessed October 2010.
15. Lee A. Lymphocytic predominance Hodgkin's lymphoma history of diseases. Pathology Case Review 2009; 8(4): 13.
16. Piris M, Gatter KC, Mason DY. CD30 expression in follicular lymphoma. Histopathology 1991; 18(1): 25–29.
17. Gutenson N, Cole P. Hodgkin lymphoma. I J Cancer 1977; 19: 595.
18. Hall PA. Immunohistochemistry in lymphoma diagnosis. BMJ 1986; 293: 1520.
19. Gruss H-J, Herrmann F. CD30 ligand, a member of the TNF ligand superfamily, with growth and activation control CD30+ lymphoid and lymphoma cells. Leuk Lymphoma 1996; 20(5-6): 397–409.