ORIGINAL RESEARCH PAPER

Pathology

IMMUNOHISTOCHEMICAL PROFILE OF HODGKIN LYMPHOMA WITH SPECIAL REFERENCE TO EBV ASSOCIATION

KEY WORDS: Hodgkin lymphoma, immunophenotype, EBV association.

Dr. P. Geetha MD, Pathology, Assistant Professor, Department Of Pathology, Government Mohan Kumaramangalam Medical College, Salem, India.

ABSTRACT

BACKGROUND: Hodgkin lymphoma is a lymphoproliferative disorder and Epstein Barr Virus infection is considered as one of the important etiological factor for Hodgkin lymphoma and prognosis depends on the clinical stage, immunological profile and EBV association.

AIMS AND OBJECTIVE: Our aim is to study the immunohistochemistry of Hodgkin Lymphoma and to correlate the expression of Epstein Barr Virus Latent Membrane Protein with histological subtypes.

50 cases of Hodgkin lymphoma were subjected to a panel of 5 immunohistochemical markers CD 15, CD30, CD20, CD45 and EBV LMP 1.

RESULT: Immunohistochemical study show predominantly Classical immunophenotype with CD 15+, CD30 + and CD 20 – constituting 77.78 %. EBV LMP positivity is present in 57% of cases, mainly in Classical type. EBV LMP1 is predominantly positive in mixed cellularity subtype.

INTRODUCTION

Hodgkin lymphoma is a lymphoproliferative disorder, accounts for 1% of all tumors[1]. Hodgkin lymphoma constitutes about 30% of all lymphomas. It was the first malignant disease defined on the basis of the histological classification of a pathognomonic giant cell (the Reed-Sternberg cell). Hodgkin lymphoma includes two types classical(CHL) and nodular lymphocyte predominant(NLPHL) in which classical type comprises four subtypes - mixed cellularity, nodular sclerosis, lymphocyte rich and lymphocyte depleted.

Epstein–Barr virus latent membrane protein 1 (LMP1) is a protein that regulates its own expression and the expression of human genes.[2] LMP1 is the best-documented oncoprotein of the EBV latent gene products.

MATERIALS AND METHODS

Total numbers of lymph node specimens received were 2961 cases, for a period of 5 years in a tertiary care hospital. Of these malignancies in lymph node accounted for 1273 cases with a percentage of 42.99%. The total number of non- neoplastic and malignant cases was 1688 and 1273 respectively. Thus the distribution of non-neoplastic lesions was 57%, and of malignant tumors were 43% among the lymph node specimens. Hodgkin lymphoma accounts for about 142 cases representing 4.8 %.

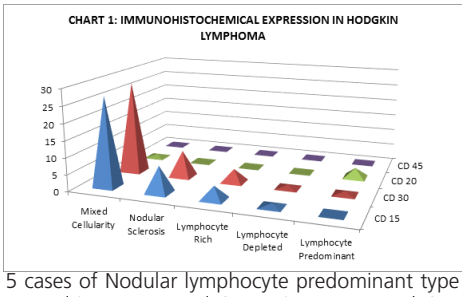
Of the 142 cases of Hodgkin lymphoma, a total of 50 cases comprising 45 cases of CHL and 5 cases of NLPHL were selected and their representative formalin fixed paraffin embedded tissue samples were subjected to immunohistochemical analysis for a panel of 5 markers- CD 15, CD30, CD20, CD45 and EBV LMP 1. Classical Hodgkin type include 29 cases of mixed cellularity, 10 cases of nodular sclerosis, 5 cases of lymphocyte rich and 1 case of lymphocyte depleted .

Immunohistochemical analysis were done in paraffin embedded tissue samples using the Next Generation Micro-Polymer HRP system based on non-biotin polymeric technology provided by Thermo Scientific Ultravision Quanto detection system for Immunohistochemistry. 4 µ thick sections from formalin fixed paraffin embedded tissue samples were transferred on to gelatin coated slides. Heat induced antigen retrieval was done.

Characteristically the tumor cells of classical type of Hodgkin lymphoma express CD30 and also positive for CD 15. The immunophenotype of nodular lymphocyte predominant is entirely different from that of classical type. The L&H cells are negative for CD15 and CD30. The markers of B cell such as CD20 and CD79a are expressed by NLPHL.

OBSERVATION AND RESULTS

Immunohistochemical expression of classical Hodgkin lymphoma shows CD 15 positivity in 40 cases and CD 30 positivity in 41 cases. Mixed cellularity subtype shows CD 15 positivity in 27 cases and CD 30 positivity in 28 cases. In nodular sclerosis subtype CD 15 and CD30 was positive in 8 cases, lymphocyte rich type shows CD 15 and CD 30 positivity in 4 cases and lymphocyte depleted was CD 15 and CD 30 positive in 1 case which was selected. CD 20 was positive in one case of mixed cellularity and negative in all cases of nodular sclerosis, lymphocyte rich and lymphocyte depleted and CD 45 was negative in all cases of classical hodgkin lymphoma(chart 1)



Out of 5 cases of Nodular lymphocyte predominant type CD 20 was expressed in 3 cases and CD 45 in 2 cases and CD 15 was negative in all cases but CD 30 positivity was seen in 1 case.

EBV LMP 1 was expressed in 26 cases of Classical Hodgkin lymphoma and none of the Nodular lymphocyte predominant type shows positivity. EBV LMP was also negative in 19 cases of classical type. There is significant association found between the type and EBV LMP expression (P value 0.014).

EBV LMP 1 was predominantly positive in mixed cellularity subtype (see Fig 1), 21 out of 29 followed by nodular sclerosis and lymphocyte rich type. Nodular sclerosis type shows 3 cases of EBV LMP positivity and was negative in 7 cases. Lymphocyte rich type was positive in 2 cases and negative in 3 cases. Single case of lymphocyte depleted subtype shows negativity for EBV LMP (chart 2).

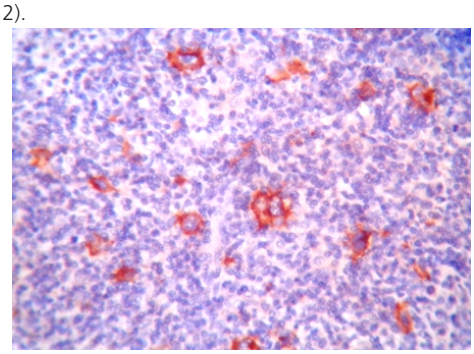
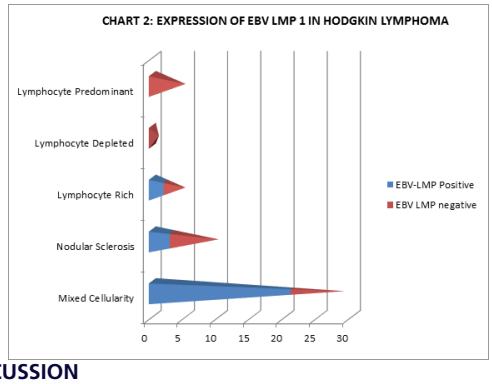


Figure 1: Mixed cellularity type showing membranous positivity for EBV Latent membrane protein 1



DISCUSSION

Hodgkin's lymphoma is the third most common cancer in people aged 15-29 years, and the sixth most commonly diagnosed cancer in children less than 14 years.

In the our study, EBV LMP immunoreactivity is seen in 57% of cases which is comparable with the study by Samia Fatima et al [3] which shows 60%. In a study conducted by Almasri et al,[4] which includes 64 cases of HL examined for EBV association, 47% immunoreactivity for EBV LMP 1 was seen, whereas Karnik[5] et al study group shows 96% positivity which is higher than our study.

EBV association is more common in mixed cellularity type of Hodgkin lymphoma which is comparable with the following studies. Almasri et al[4] study shows 52.9% positivity, Samia Fatima et al[3] study shows 71% positivity for mixed cellularity and also 54% positivity for nodular sclerosis, Quintanilla et al[6], Weinreb et al [7],Murray et al [8],Monterroso et al [9] and Peh et al [10] studies show 81%, 85%, 86% and 87% positivity for mixed cellularity respectively. Our current study shows 80.7 % positivity for mixed cellularity and 11.5% positivity in nodular sclerosis subtype. All the above studies shows 15% to 50% positivity for nodular sclerosis subtype.

In this study, the immunohistochemical expression of cases can be divided into four groups which carries prognostical significance. Group I which includes 35 cases was positive for CD 15 and CD 30 and negative for CD 20. Group II includes 5 cases which was positive for CD 30 alone and negative for CD 15. Group III category shows 4 cases which was positive for CD 15 alone and negative for CD 30. Group IV includes 1 case which was positive for CD 20.

This is comparable to the study by Patkar N et al [11] in which the cases are divided similarly which shows higher percentage of classical immunophenotype ie CD 15+, CD 30+ and CD 20-

Negative expression of CD 15 and immunoreactivity for CD 20 is an adverse prognostic factor.

CONCLUSION

Among 50 cases, 35 cases have classical immunophenotype (CD 15+, CD 30+ and CD 20-). EBV association is seen in 26 cases with statistically significant association seen between the type of HL and EBV infection.

Immunohistochemical study show predominantly Classical immunophenotype with CD 15+, CD30 + and CD 20 – constituting 77.78 %. EBV LMP positivity is present in 57% of cases and seen only in Classical type and not seen in Nodular lymphocyte predominant type and there is statistically significant association found between the type of Hodgkin lymphoma and Epstein Barr virus association. EBV LMP1 is predominantly positive in mixed cellularity subtype.

To conclude, identifying the classical immunophenotype is very important which have better prognosis whereas CD15- and CD 20+ are considered as adverse prognostic parameters. Identifying the EBV association is very significant because Reed Sternberg cells present in EBV associated cases show a better response to chemotherapy and EBV association provides survival advantage to hodgkin lymphoma patients with standard chemotherapy and radiotherapy protocols and is an excellent candidate for targeted cellular immunotherapy.

REFERENCE

1. Pier Paolo Piccaluga, Claudio Agostinelli, Anna Gazzola, Claudio Tripodo,Francesco Bacci, Elena Sabattini, Maria Teresa Sista, ClaudiaMannu,Maria Rosaria Sapienza, Maura Rossi, Maria Antonella Laginestra,Review Article.Pathobiology of Hodgkin Lymphoma .Advances in HematologyVolume 2011, Article ID 920898, 18 pages.
2. Pratt, Z; Zhang, J; Sugden, B. (2012). "The latent membrane protein 1 (LMP1) oncogene of Epstein–Barr virus can simultaneously induce and inhibit apoptosis in B cells". Journal of Virology 86 (8): 4380–4393.
3. Samia Fatima, Rashida Ahmed, Arsalan Ahmed. Hodgkin Lymphoma in Pakistan: An Analysis of Subtypes and their Correlation with Epstein Barr Virus. Asian Pacific J Cancer Prev, 12, 1385-1388.
4. Nidal M. Almasri,, Hasan S. Khalidi. Epstein Barr virus Expression in Hodgkin's disease in Jordan. Saudi Med J 2004; Vol. 25 (6): 770-775.
5. Karnik S, Srinavasan B, Nair S (2003). Hodgkin's lymphoma: Immunohistochemical features and association with EBV LMP-1 from a South Indian Hospital. Pathology, 35, 207-11.
6. Quintanilla-Martinez L, Gamboa-Domnquez A, Gamez-Ledesma I, Angeles-Angeles A, Mohar A. Association of Epstein-Barr virus latent membrane protein and Hodgkin's disease in Mexico. Mod Pathol 1995; 8: 675-679.
7. Weinreb M, Day PJ, Murray PG, Raafat F, Crocker J, Parkes SE, et al. Epstein-Barr virus (EBV) and Hodgkin's disease in children: incidence of EBV latent membrane protein in malignant cells. J Pathol 1992; 168: 365-369.
8. Murray PG, Young LS, Rowe M, Crocker J. Immunohistochemical demonstration of the Epstein-Barr virus-encoded latent membrane protein in paraffin sections of Hodgkin's disease. J Pathol 1992; 166: 1-5.
9. Monterroso V, Zhou Y, Koo S, Glackin C, Bujan W, Medeiros LJ. Hodgkin's disease in Costa Rica: a report of 40 cases analyzed for Epstein-Barr virus. Am J Clin Pathol 1998; 109: 618-624.
10. Peh SC, Looi LM, Pallesen G. Epstein-Barr virus (EBV) and Hodgkin's disease in a multi-ethnic population in Malaysia. Histopathology 1997; 30: 227-233.
11. Patkar N, Mehta J, Kulkarni B, et al. Immunoprofile of Hodgkin's lymphoma in India. Indian Journal of Cancer 2008;45(2).