



ORIGINAL RESEARCH PAPER

Pathology

IMMUNOHISTOCHEMICAL ANALYSIS OF NON HODGKIN'S LYMPHOMAS WITH SPECIAL REFERENCE TO EPSTEIN –BARR VIRUS

KEY WORDS: NHL, LMP, Epstein Barr virus

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ABSTRACT

Epstein–Barr Virus(EBV) is a herpes virus and EBV positive tumour cells express latent membrane protein-1(LMP-1), a protein encoded by EBV genome that has transforming activity. EBV virus infects B-lymphocytes, which will emerge as lymphomas .

Aim: To demonstrate EBV antigen in tissue sections of Non Hodgkin's lymphomas (HL) by immunohistochemistry (IHC) and to investigate the relationship between EBV associated NHL in view of age, site and subtype

Material And Methods: 39 cases of Non Hodgkin's lymphomas were taken for histopathological examination & IHC marker LMP-1 study.

Conclusion: Out of 39 cases 9 show positivity and LMP-1 expression is more common in elderly age group, diffuse large B cell lymphoma subtype and in CNS Lymphomas. Factors such as age, site, histological subtype, & immunity of the individuals are modifiers of EBV influence on pathogenesis of this neoplasm

Introduction

Lymphomas are a heterogenous group of malignant neoplasms, which originate primarily in lymph nodes or other lymphoid tissues due to monoclonal proliferation of lymphocytes that arise as discrete tissue masses. Lymphomas are divided in to two major types as Hodgkin's lymphoma (HL) and Non Hodgkin's lymphoma (NHL). Burkitt's lymphoma, primary central nervous system lymphomas(PCNSL), Diffuse large B cell lymphomas in elderly, cutaneous lymphomas, T cell/NK cell lymphoproliferative disease, Lymphomas of primary immunodeficiencies, post transplant lymphoproliferative disorder and AIDS related lymphomas are the NHL's that are associated with EBV.

Cd21⁺ B-lymphocytes can easily be infected with EBV in vitro, where upon they become growth transformation in to lymphoblastoid cell lines (LCLs) with infinite proliferative potential resulting in lymphomas .

Latently infected B cells express a limited number of EBV genes. They are 3 membrane proteins: LMP:-1, LMP-2A, and LMP-2B &.6 Nuclear proteins: EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C and EBNA-LP(Leader protein). EBV encoded small RNAs:- EBER 1 and EBER 2. EBV expression in Non Hodgkin's lymphoma affects the prognosis and long term survival in view of age ,sex and subtype. In Non Hodgkin's lymphoma VEGF-A is the essential factor in tumour angiogenesis. There is evidence of cross talk between angiogenesis and viral carcinogenesis. The viral latent protein LMP-1 may play a role by inducing expression of angiogenic factors. EBV positivity was associated with VEGF-A expression in diffuse large B cell lymphoma (DLBCL) and a shorter survival rate. Advanced age and stage adversely affect the outcome in NHL .

The study had been carried out in the department of pathology, GMKMC, Salem during the period of June 2011- May 2014. A total number of 39 cases which were already diagnosed as Non Hodgkin's lymphomas by histopathological examination and proved as NHL by IHC were taken for EBV study by using the immunohistochemical marker LMP-1.10% buffered neutral formalin were used for fixation. The tissues were processed, paraffin blocks were prepared, 5 micron thin sections were cut by using automatic microtome and stained with hematoxylin and eosin. Suitable sections were chosen for IHC. Slides were coated with poly- L- lysine, and subjected to antigen retrieval using the Microwave technique with TRIS – EDTA buffer solution. Slides were then treated by HRP (Horse radish peroxidase) polymer technique. LMP-1 (CS 1-4 antibody Dako cytomaton) was used in all 39 cases of Non Hodgkin's lymphoma.

Observation & Results

Out of 39 cases LMP-1 expressivity is more commonly seen in elderly, Primary central nervous system lymphomas and diffuse large B cell lymphoma subtype & in imunocompromised

individuals. In our study a total number of 9 cases out of 39 cases show positivity for LMP-1.

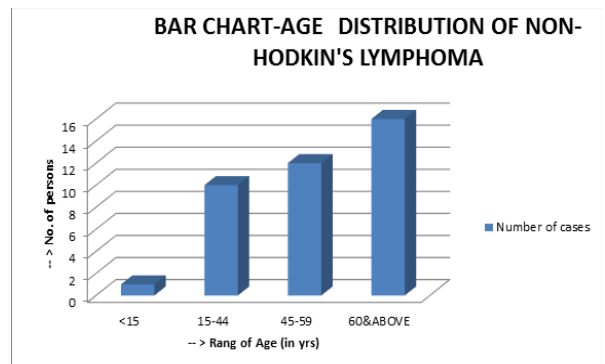


TABLE-1 LMP 1 EXPRESSION IN DIFFERENT AGE GROUPS OF NON HODGKIN'S LYMPHOMA

S.NO	AGE	TOTAL (No of cases)	LMP1 positivity	%
1	<15	1	0	0
2	15-44	10	1	10
3	45 -59	12	2	16.7
4	60&above	16	6	37.5

TABLE-2 SITE DISTRIBUTION OF NON HODGKIN'S LYMPHOMA

S.No	SITE	NO.	LMP-1 Positivity	%
1	CNS Lymphomas	6	5	83.33
2	Cutaneous Lymphomas	3	1	33.33
3	Gastrointestinal Lymphomas	9	2	22.22
4	Tonsil	2	0	0
5	Retroperitoneum	2	0	0
6	Mediastinum	1	1	0
7	Nasopharynx	1	0	0
8	Cervical lymphnodes	15	0	0

TABLE-3 SUBTYPE DISTRIBUTION OF NON –HODGKIN'S LYMPHOMA

S.NO	SUBTYPE	NO	%
1	DIFFUSE LARGE B CELL LYMPHOMA	34	87.18
2	SMALL LYMPHOCYTIC LYMPHOMA	1	2.56
3	LYMPHOBLASTIC LYMPHOMA	1	2.56
4	FOLLICULAR LYMPHOMA	1	2.56
5	MALTOMA	2	5.13
	TOTAL	39	100

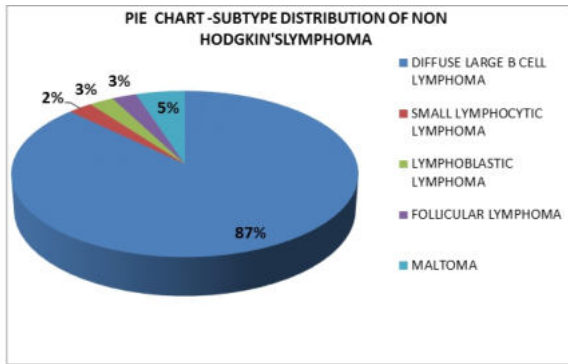


TABLE-3 LMP-1 EXPRESSION IN SUBTYPES OF NON HODGKIN'S LYMPHOMA

S. NO	SUBTYPE	NO	LMP-1 Positivity	%
1	DIFFUSE LARGE B CELL LYMPHOMA	34	8	23.53
2	SMALL LYMPHOCYTIC LYMPHOMA	1	0	0
3	LYMPHOBLASTIC LYMPHOMA	1	1	100
4	FOLLICULAR LYMPHOMA	1	0	0
5	MALTOMA	2	0	0

DISCUSSION

Several lines of evidence have suggested that NHL is related to EBV. Primary central nervous system lymphoma (PCNSL-NHL) is reserved for malignant lymphoid neoplasm restricted at presentation to the brain, spinal cord or meninges.¹ Most PCNSL are high grade NHL of diffuse large B cell type² rarely signet ring cell type and anaplastic large cell lymphoma. The overwhelming majority of PCNSLs exhibit a B cell immunophenotype. The increased incidence of PCNSL is likely due to its occurrence among immunosuppressed patients and in patients with AIDS³. The association of EBV with AIDS-related PCNSL varies from 75%-100% in different studies.

PCNSL that arise in the immunocompromised individuals produce a distinctly poorer prognosis than those in the immunocompetent.⁴ In case of primary CNS lymphomas Murphy et al study showed 11/24(46%) cases of EBV LMP-1 in immunocompetent PCNSL individuals.⁵ Aswani tandam et al study showed 80% positivity in immunocompetent PCNSL individuals⁶. Our study shows a total number of 6 cases of PCNSL-NHL & all are reported as Diffuse large B cell lymphomas. Out of 6 cases of PCNSL, 5 cases show LMP-1 expressivity (83%).

Patients with age-related EBV positive B-cell lymphoproliferative disorder showed a higher age distribution and aggressive clinical features than EBV negative diffuse large B cell lymphomas.⁷ Senile EBV positive lymphoproliferative disorder is a form of EBV positive large B cell lymphoma that occurs in elderly patients over 60 years without evidence of immunodeficiency. These lymphomas are related to the immunological deterioration that occurs during the aging process⁸. The prognosis of polymorphic type of EBV positive lymphoma is much better than monomorphic EBV positive lymphoma in elderly⁹.

In this study 16/39(41%) cases were diffuse large B cell lymphomas in elderly and were above the age of 60. Out of this 6/16 cases(37.5%) were positive for EBV LMP-1. Several studies states that Shimoyoma et al(37%), Oyama T et al(40%) states that there was a significant association with EBV in case of Diffuse large B cell lymphoma of elderly. This suggests a bigger role for EBV in the pathogenesis of this kind of lymphomas and warrants studies on a larger sample size.

In case of AIDS related lymphoproliferative disorder Aswani Tandon et al study showed 100% EBV LMP-1 positivity in primary central nervous system lymphomas in AIDS patients. In the present study 4(all PCSNL) out of 39 cases were HIV positive cases and in all 4 cases (4/4) were LMP-1 Positivity.(100%).

Expression of EBV genome appears to be seen in T-cell lymphomas. Both EBNA 2 and LMP-1 have been detected in addition to EBNA-1¹⁰. Out of 39 cases, one case which was presented as mediastinal mass was reported as Lymphoblastic lymphoma of T cell type and was positive for CD-3 & LMP-1 expression. Worldwide EBV association is seen in 10-35% of T / NK cell lymphoma and associated with poor prognosis^{11,12}.

In cutaneous Non Hodgkin's lymphoma the finding of strong EBER-RNAs and LMP-1 expression in a patient with apparent skin-limited lesions suggests that there is a serious probability of systemic disease. Thus, such a finding should be an important indicator of poor prognosis¹³. Primary cutaneous large B-cell lymphoma (PCLBCL) is a subtype of lymphoma with a female predominance, occurring in elderly patients and known to have unfavourable prognosis¹⁴. In our present study 3 out of 39 cases were cutaneous NHL, and out of this 1 was positive for LMP-1.

Burkitt's lymphomas harbor EBV genomes. Type I latency is seen in Burkitt's lymphoma. In Burkitt's lymphoma only EBNA1 and EBER are the latent gene products expressed and the LMP-1 and other latent genes expression are downregulated¹⁵.

Post transplant lymphoproliferative disease (PTLD) contributes to 12% of post transplant malignancies and its incidence increases with time. 80-90% of PTLD cases are associated with primary EBV infection or reactivation of previously acquired EBV. There were no cases reported as Burkitt's lymphoma, X linked lymphoproliferative disorder and post transplant lymphoproliferative disorder in the present study.

VI. CONCLUSION

Increased expression of LMP -1 was observed in elderly age group (60& above), Primary central nervous system lymphomas, immune compromised individuals and majority of them were Diffuse large B cell lymphomas. Among different studies suggests that factors of age, site, histological subtype & immunity of the individuals are modifiers of EBV influence on pathogenesis of this neoplasm. Future work to identify the role of latent viral products in association with EBV associated Non Hodgkin's lymphoma is required. Such knowledge is likely to pave the way for greater refinement of EBV targeted gene therapy.

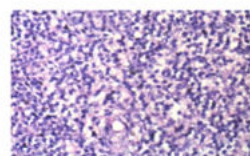


Fig.1 CNS spinal extra dural SOL, NHL-diffuse large B-cell lymphoma H&E (40X)

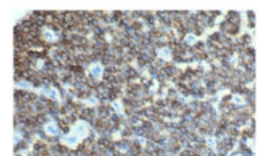


Fig.2 CNS spinal extra dural SOL, NHL-diffuse large B-cell type lymphoma-LMP-1 positive (40X)

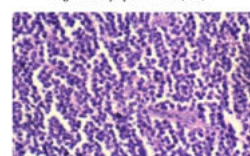


Fig.3 NHL lymphoblastic lymphoma H & E (40X)

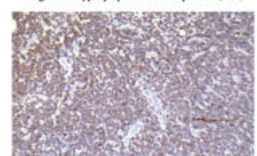


Fig.4 NHL lymphoblastic lymphoma-CD3 Positive(10 x)

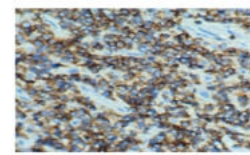


Fig.5 NHL lymphoblastic lymphoma (T-cell type) LMP1 positive (40X)

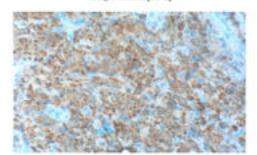


Fig.6 NHL-Diffuse large B cell lymphoma of elderly (gastrointestinal tract) - LMP-1 positive(40X)

VII. References

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