ORIGINAL ARTICLE

EPSTEIN BARR VIRUS LMP-1 POSITIVITY IN HODGKIN LYMPHOMA SUBTYPES, IN KHYBER PAKHTUNKHWA PROVINCE OF PAKISTAN

Sara Ziaullah, Sajjad Ahmad, Muhammad Mumtaz Khan, Sadaf Alam, Sabeen Nasir, Naveed Sharif

Peshawar Medical College, Peshawar, Riphah International University, Islamabad, Pakistan

ABSTRACT

Background: Epstein Barr Virus (EBV) is commonly associated with Hodgkin Lymphoma. Our aim was to detect Epstein Barr virus LMP-1 antigen in subtypes of Classical Hodgkin Lymphoma in Peshawar district of Khyber Pakhtunkhwa Province of Pakistan.

Material & Methods: This cross sectional study was carried out in Peshawar Medical College (PMC), Peshawar and Pakistan Institute of Medical Sciences (PIMS) Islamabad. It included 50 cases of Hodgkin lymphoma diagnosed on lymph node biopsy from August 2007 to August 2013. Hematoxylin and eosin stain for light microscopy was done at PMC. Immunohistochemistry for EBV was performed at PIMS, Islamabad. Demographic variables were gender, age in years and age groups. Research variables were; HL subtypes and EBV LMP-1 antigen status. Age in groups had 4 attributes of $\leq$15,16-34, 35-49 and $\geq$50 years. EBV had 2 attributes of positive and negative. All variables were categorical except age in years and were expressed as frequency and percentages , whereas age in years (numeric) as mean and SD. Statistical analysis was carried out using SPSS version 19. The difference between positive and negative EBV LMP1 receptor status were analyzed for statistical significance using Fischer's exact test. Probability value $p \leq 0.05$ was considered statistically significant.

Results: The mean age of patients was 27.12 years with a male predominance 33 (66%). Mixed Cellularity was the commonest type 31 (62%) followed by Nodular Sclerosis 12 (24%), Immunohistochemical (IHC) staining for EBV antigen was positive in 40 (80%) of cases. The maximum number of positivity was seen in Mixed Cellularity type 29 (72.5%) followed by Nodular Sclerosis 9 (22.5%). The p-value was significant for EBV antigen positivity in the subtypes of Hodgkin Lymphoma.

Conclusion: EBV is a major risk factor for Mixed Cellularity and Nodular Sclerosis subtypes of Hodgkin Lymphoma in KPK Province of Pakistan.

KEY WORDS: Hodgkin lymphoma; EBV; Reed-Sternberg cells.

This article may be cited as: Ziaullah S, Ahmad S, Khan MM, Alam S, Nasir S, Sharif N. Epstein barr virus lmp-1 positivity in hodgkin lymphoma subtypes, in Khyber Pakhtunkhwa province of Pakistan. Gomal J Med Sci 2017;15:3-7.

INTRODUCTION

Hodgkin lymphoma (HL) is characterized by the presence of neoplastic giant cells called Reed-Sternberg cells. These cells release factors that induce the accumulation of reactive lymphocytes, macrophages, and granulocytes, which typically make up greater than 90% of the tumor cellularity. In the vast majority of HLs, the neoplastic Reed-Sternberg cells are derived from germinal center or post germinal center B cells. The WHO classification recognizes five subtypes of HL, viz., Nodular Sclerosis (NS), Mixed Cellularity (MC), Lymphocyte Rich (LR), Lymphocyte Depletion (LD) and Lymphocyte Predominance (LP). Based on immunophenotype of the neoplastic cells the first four are grouped together as Clinical Hodgkin Lymphoma.[1]

HL constitutes about 0.5% of cancer worldwide with an Age Standardized Rate (ASR) of 0.9/100, 000. Asians generally have low incidence of HL as compared to Europeans. In many parts of Asia ASR of Hodgkin Lymphoma is 0.6/100,000, whereas ASR of European region is 2.1/100, 000.[2]Variation between countries may reflect different prevalence of risk factors, use of screening and diagnostic methods.

In Pakistan, Shaukat Khanum Memorial Cancer

Corresponding Author:

Dr. Sara Ziaullah
Peshawar Medical College
Peshawar, Pakistan
E.mail: drsarazia@gmail.com

Date Submitted: 06-10-2016
Date Revised: 17-02-2017
Date Accepted: 20-02-2017

Hospital and Research Centre, Lahore (SKMCH & RC) mainly covers provinces of Punjab and Khyber Pakhtunkhwa. According to its collective cancer registry report from December 1994 to December 2014, HL is the 7th most common cancer, whereas in pediatric population (< 18 yrs) it is the 2nd most common malignancy constituting 4.36% of all malignancies in all age groups and about 18.98% of all malignancies in age group < 18 years.[3]

According to a report from Agha Khan University Hospital (AKUH), Karachi, Pakistan which mainly covers provinces of Sindh and Baluchistan in the south, HL ranks as 10th most common neoplasm in males while 14th in females.[4,5] In another study at AKUH in the pediatric population (age 1-10 yrs) HL is the most common cancer in males (25.92%) while 3rd most common cancer in females (11.47%).[5]

HL in different age groups has different etiologies and infection by EBV has been strongly implicated.[6] After reviewing accumulated patient data from 14 studies it was suggested that age, sex, ethnicity and the physiologic effects of poverty may represent biologic modifiers of the EBV association and confirm that this association is strongly but variably linked to histologic subtype. The data augmented biologic evidence that EBV is actively involved in HL pathogenesis in some cases but describe epidemiologic complexity in this process.[7] EBV strains type 1 and type 2 show a significant variation in different geographical regions and age groups.[8] In Pakistan the clinico-epidemiological pattern of Hodgkin's lymphoma is similar to other developing countries, with male predominance, mixed cellularity as the commonest histological type, advanced stage at presentation and absence of bimodal age distribution.[9]

Regarding EBV positivity in Pakistan a study carried out at AKUH, Karachi, speculated about prognostic effects of EBV infection on the course of HL and hoped that the EBV-positive HL could in the future prove to be an excellent candidate for targeted cellular immunotherapy.[10] There is scarcity of work done on EBV associated HL in Pakistan, therefore, the aim of this study was to detect EBV antigen in CHL in Peshawar District, Khyber Pakhtunkhwa Province of Pakistan.

MATERIALS AND METHODS

This cross sectional study was carried out in Peshawar Medical College (PMC), Peshawar and Pakistan Institute of Medical Sciences (PIMS) Islamabad. It included 50 cases of Hodgkin lymphoma (HL) diagnosed on lymph node biopsy from August 2007 to August 2013. Hematoxylin and eosin (H&E) stain for light microscopy was done at PMC. Immunohistochemistry (IHC) for EBV was performed at PIMS, Islamabad. Demographic variables were gender, age in years and age groups. Research variables were; HL subtypes and EBV LMP-1 antigen status. Age in groups had 4 attributes of ≤15,16-34, 35-49 and ≥50 years. EBV had 2 attributes of positive and negative. All variables were categorical except age in years and were expressed as frequency and percentages , whereas age in years (numeric) as mean and SD. Statistical analysis was carried out using SPSS version 19.

The difference between positive and negative EBV LMP-1 receptor status were analyzed for statistical significance using Fischer's exact test. Probability value $p \leq 0.05$ was considered statistically significant.

RESULTS

Clinical findings

There were 33 (66%) males and 17 (34%) females. Their age ranged between 5 to 80 years with a mean of 27.12 years (SD ±19.14). Of these cases 17 (34%) belonged to pediatric age group ≤15 years, 16 (32%) to age group 16-34, 10 (20%) to age group 35-49, and 7 (14%) to ≥50 years.

Histopathological findings

All the 50 cases were confirmed IHC as HL using a panel of monoclonal antibodies (CD15, CD20, CD30 and CD45). The maximum number of cases of HL were of MC type 31 (62%) followed by NS 12 (24%) and LR type 3 (6%). Two cases each (4%) belonged to LD and LP. In all the age groups MC was the most common lesion followed by NS in the age groups ≤15 and 16-34 years. (Table 1).

IHC staining for EBV LMP-1 antigen was positive in 80% cases and negative in 20% cases. The EBV LMP-1 status among gender, in various age groups and in HL subtypes (Table 2).

Table 1: Distribution of Hodgkin Lymphoma subtypes in various age groups.

Age group	MC (%)	NS (%)	LR (%)	LD (%)	LP (%)	Total (%)
≤ 15	11(64.7)	5(29.4)	0	0	1(5.8)	17(34)
16-34	8(50)	6(37.5)	1(6.25)	1(6.25)	0	16(32)
35-49	7(70)	0	2(20)	1(10)	0	10(20)
> 50	5(71.4)	1(14.2)	0	0	1(14.2)	7(14)
Total	31	12	3	2	2	50

Table 2: Distribution of EBV positivity in relation to gender, age and subtypes of HL.

	Cases (n=50)		EBV+ (n=40)		EBV- (n=10)		p- Value
Gender	**No.**	**%**	**No.**	**%**	**No.**	**%**	
Male	33	66	27	67.5	6	60	0.7172
Female	17	34	13	32.5	4	40	
Age groups							
≤ 15	17	34	15	37.5	2	20	0.7331
16-34	16	32	10	27.5	6	60	
35-49	10	20	9	22.5	1	10	
≥ 50	7	14	6	15	1	10	
Sub types							
MC	31	62	29	72.5	2	20	0.0096
NS	12	24	9	22.5	3	30	
LR	3	6	1	2.5	2	20	
LD	2	4	1	2.5	1	10	
LP	2	4	0	0	2	4	

DISCUSSION

This discussion includes EBV positivity in relation to age, sex and HL subtypes especially MC and NS.

In developed countries, several studies point out that in younger age group HL occurs predominantly in males. In a study of children with HL in North India there was a remarkably high percentage of males (89.7%) as compared to females (10.3%)[11] and to somewhat variable degrees this male predominance is reflected in our study as well as in a Taiwanese [12] and in a Turkish study.[13] The mean age in the Taiwanese study was 42 years which is quite higher than ours although the age range was comparable which reflected that our population was involved by HL at a younger age.[12]

Histologically the predominant HL subtype in our study in all age groups was MC followed by NS which is identical to other national studies.[10, 14] However, this is in contrast to the data obtained from Middle East [15, 16] and Europe [17] where NS follows MC.

EBV LMP-1 in HL in our study showed positivity in children with a male predominance which is in conformity with other studies.[16]

In our study the EBV LMP-1 positivity was maximally shown in the MC subtype (72.5%) followed by NS (22.5%), LR (2.5%) and LD (2.5%). At the national level in a study carried out in Karachi, Pakistan EBV LMP-1 showed strongest positivity association with MC subtype (71%) followed by NS (54.2%) and children showed more positivity (87.1%) as compared to adults (49.3%).[10] The identical EBV LMP-1 positivity association is found in our study, i.e., MC followed by NS with more positivity in the age group ≤15 years. In a Jordanian study maximum EBV positivity was found in age groups < 15 yrs (68%) and > 51 years (78%) [18] which in our study stands true for the ≤ 15 years age group (37.5%) but in contrast to the age group ≥ 50 years (15%).

In the North Indian study the MC subtype (72.4%) was much higher than NS (22.7%) but both showed identical EBV positivity (97%).[11] In contrast to this in a South Indian study EBV LMP-1 showed more positivity in the NS subtype, 86% cases.[19]

In the Middle East in Jordan where the NS constituted 70% of all HL in a study, the maximum positivity was found in MC subtype (80.7%) as compared to NS (30.3%).[18] Similarly in a study of EBV associated HL in Cairo, Egypt, the NS subtype constituted 84% but showed EBV positivity for 60% in contrast to MC constituting 9% with 100% EBV positivity.[20] In Iraq MC subtype (42%) dominated NS (22%) but again the MC was 100% positive for EBV LMP-1 as compared to 90.9% positivity for NS.[21] In the Iran NS (66%) dominated over MC (31%) but LMP-1 was positive in 46.7% cases of MC as compared to 15.4% of NS and the difference was statistically significant.[22]

In an Austrian study NS was the most common subtype with 59% cases, followed by 31% cases of MC subtype but the EBV positivity by LMP-1 immuno-reactivity was detected in 21% of the NS subtype and 38% of the MC subtype.[17] In Latin America in a retrospective study of EBV associated HL among children in Argentina, MC subtype showed 76% positivity whereas all cases of NS were negative. There was maximum positivity in the age group 1-6 years

(82%) which declined to 33% in the age group 7-15 years.[23]

In a study carried out in Northern China, the EBV positivity was more in MC subtype (71%) as compared to NS (25%).[24] In a South African study although 89% of children had NS subtype as compared to 11% MC but showed 80% EBV positivity for MC against 67% of NS.[25]

The above discussion points at almost universal positivity of MC as compared to NS subtype even at places where the latter dominated except in a South Indian study where NS showed more positivity for EBV LMP-1.[19] The picture is also different in a study from Northern Iraq where NS (58.5%) was more than MC (37.2%) and LD (2.8%) but the maximum EBV LMP-1 positivity was shown in LD (50%) as compared to MC (45%) and NS subtype (27.7%).[16]

No significant relationship of EBV positivity could be found in relation to age and gender in our study. However, a statistically significant relationship of EBV positivity with subtypes of classical HL (p value 0.0096) was detected.

CONCLUSION

The present study revealed that EBV is a major risk factor for HL in KPK province of Pakistan, especially in the MC and NS subtypes.

REFERENCES

1. Kumar V, Abbas AK, Aster JC. In Diseases of White Blood Cells, Lymph Nodes, Spleen, and Thymus. Edited by Kumar V, Abbas AK, Aster JC. Philadelphia, Elsevier Saunders, 2015, p. 607
2. Estimated cancer incidence, mortality and prevalence worldwide. Edited by WHO, International Agency for Research on Cancer, 2012, p.
3. Mahmood S, Faraz R, Yousaf A, Asif H, Badar F. Collective cancer registry report from Shaukat Khanum Memorial Cancer Hospital and Research Center (SKMCH & RC) Dec. 1994 till Dec. 2014;2015, 1-11.
4. Ahmad Z, Azad NS, Yaqoob N, Husain A, Ahsan A, Khan AN, et al. Frequency of primary solid malignant neoplasms in both sexes, as seen in our practice. J Ayub Med Coll Abbottabad 2007;19:53-5.
5. Ahmed Z, Azad NS, Rauf F, Yaqoob N, Husain A, Ahsan A, et al. Frequency of primary solid malignant neoplasms in different age groups as seen in our practice. J Ayub Med Coll Abbottabad 2007;19:56-63.
6. Thomas RK, Re D, Zander T, Wolf J, Diehl V. Epidemiology and etiology of Hodgkin's lymphoma. Euro Society Med Oncol 2002;13:147-52.
7. Glaser SL, Lin RJ, Stewart SL, Ambinder RF, Jarrett RF, Brousset P, et al. Epstein-Barr virus-associated Hodgkin's disease: epidemiologic characteristics in international data, Int J Cancer 1997;70:375-82.
8. Weinreb M, Day PJ, Niggli F, Powell JE, Raafat F, Hesseling PB, et al. The role of Epstein-Barr virus in Hodgkin's disease from different geographical areas, Arch Dis Child 1996;74:27-31.
9. Siddiqui N, Ayub B, Badar F, Zaidi A. Hodgkin's lymphoma in Pakistan: a clinico-epidemiological study of 658 cases at a cancer center in Lahore. Asian Pac J Cancer Prev 2006;7:651-5.
10. Fatima S, Ahmed R, Ahmed A. Hodgkin lymphoma in Pakistan: an analysis of subtypes and their correlation with Epstein Barr virus. Asian Pac J Cancer Prev 2011;12:1385-8.
11. Dinand V, Dawar R, Arya LS, Unni R, Mohanty B, Singh R. Hodgkin's lymphoma in Indian children: prevalence and significance of Epstein-Barr virus detection in Hodgkin's and Reed-Sternberg cells. Eur J Cancer 2007;43:161-8.
12. Liu SM, Chow KC, Chiu CF, Tzeng CH. Expression of Epstein-Barr virus in patients with Hodgkin's disease in Taiwan. Cancer 1998;83:367-71.
13. Yilmaz F, Uzunlar AK, Sogutcu N, Ozaydin M. Hodgkin's disease and association with Epstein-Barr virus in children in Southeast Turkey. Saudi Med J 2005;26:571-5.
14. Mushtaq S, Akhtar N, Jamal S, Mamoon N, Khadim T, Sarfaraz T, et al. Malignant lymphomas in Pakistan according to the WHO classification of lymphoid neoplasms. Asian Pac J Cancer Prev 2008;9:229-32.
15. Nagi AH, Al-Menawy LA, Samiullah, Naveed IA, Sami W. A comparison of histological appearances of Hodgkin's disease in Pakistani and Saudi patients. J Ayub Med Coll Abbottabad 2008;20:66-9.
16. Saeed MS. Epstein-Barr virus in Hodgkin's lymphoma-immunohistochemical case series study. Ann Coll Med Mosul 2009;35:93-103.
17. Krugmann J, Tzankov A, Gschwendtner A, Fischhofer M, Greil R, Fend F, et al. Longer Failure-Free Survival Interval of Epstein-Barr Virus-Associated Classical Hodgkin's Lymphoma: A Single-Institution Study. Mod Pathol 2003;16:566-73.
18. Sughayer MA, Haddad HA, Al-Yousef RM, El-Khateeb M, Abu-Rass H. Epstein-Barr virus and Hodgkin lymphoma in Jordan. Hematol Oncol Stem Cell Ther 2014;7:85-9.
19. Karnik S, Srinivasan B, Nair S. Hodgkin's lymphoma: immunohistochemical features and its association with EBV LMP-1. Experience from a South Indian hospital. Pathol 2003;35:207-11.
20. Audouin J, Diebold J, Nathwani B, Ishak E, MacLennan K, Mueller-Hermelink HK, et al. Epstein-Barr virus and Hodgkin's lymphoma in Cairo, Egypt. Edited by J Hematop 2010;3:11-8.
21. Al-Mudallal SS, Al-Sinjery GM. Immunohistochemical Expression of Epstein Barr Virus Antigen Latent Membrane Protein-1 and Bcl-2 in Classical Hodgkin Lymphoma. Iraqi J Med Sci 2012;10:234-42.
22. Rajabi M, Rajabi P, Eftekhari A, Eftekhari M,

Ghasemibasir H, Ali. The effect of Epstein-Barr virus Latent Membrane Protein-1 status on outcome of patients with classic Hodgkin`s Lymphoma. Pak J Med Sci 2008;24:531-6.

23. Preciado M, De Matteo E, Diez B, Menarguez J, Grinstein S. Presence of Epstein-Barr virus and strain type assignment in Argentine childhood Hodgkin's disease. Blood 1995;86:3922-9.

24. Huang X, Nolte I, Gao Z, Vos H, Hepkema B, Poppema S, et al. Epidemiology of classical Hodgkin lymphoma and its association with Epstein Barr virus in Northern China. PLoS One 2011;6:1-10.

25. Engel M, Essop MF, Close P, Hartley P, Pallesen G, Sinclair-Smith C. Improved prognosis of Epstein-Barr virus associated childhood Hodgkin's lymphoma: study of 47 South African cases. J Clin Pathol 2000;53:182-6.

CONFLICT OF INTEREST
Authors declare no conflict of interest.
GRANT SUPPORT AND FINANCIAL DISCLOSURE
None declared.

AUTHORS' CONTRIBUTION

Conception and Design:	SZ, SA, MMK
Data collection, analysis & interpretation:	SZ, SA, SN, NS
Manuscript writing:	MMK, NS