



DETECTION OF EPSTEIN - BARR VIRUS IN LYMPHOMA LESIONS AMONG SUDANESE PATIENTS USING LMP-1 MONOCLONAL ANTIBODY

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ABSTRACT

OBJECTIVE: this was a retrospective descriptive study aimed to investigate the association of EBV with different types of lymphoma in Sudanese patients.

METHODS: Fifty paraffin-embedded formalin-fixed tissue sections of lymphoma lesions were immunohistochemically stained using latent membrane protein-1 (LMP-1) Monoclonal Antibody and results were analyzed using SPSS version 16.

RESULTS: Only two cases were positive for EBV LMP-1, one was NHL B cell type and the other was Hodgkin's Lymphoma.

CONCLUSION: the results do not support considering EBV LMP 1 as a significant prognostic factor for lymphoma in our locality.

KEY WORDS: EBV, LMP-1, Lymphoma, Sudan

INTRODUCTION:

Lymphoma is the cancer of the immune cells, lymphocytes. There are two types of lymphoma: Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL). Hodgkin's lymphoma originates from B lymphocytes while NHL originates from either B or T lymphocytes. According to the American Cancer Society, Hodgkin lymphoma was 11.8% of all types of lymphoma diagnosed in 2013 in the United States, while NHL is considered the sixth most common cancer in males and the seventh in females [1].

In Sudan, few studies have been done on lymphoma. Salwa HS [2] studied 1205 malignant tumors; of them 5.4% were NHLs. Intisar E. Saeed et al [3] analyzed 6771 new cancer cases registered during 2009–2010 in Khartoum and reported that lymphoma was the third cancer in men and the second in children; the calculated incidence rate of lymphoma was 8.2 per 100,000 population.

Leukemia and lymphoma are etiologically closely related to viruses [4]. Infection of adults by the herpes virus Epstein-Barr virus (EBV) is a worldwide problem [5]. The EBV is characterized by its affinity to lymphoid cells [6, 7]. It is estimated that about 30-40 percent of patients with Hodgkin's lymphoma in the Western world and in some developing regions carry EBV in the malignant Hodgkin Reed Sternberg (HRS) cells [8, 9, and 10].

Epstein-Barr virus latent membrane protein 1 (LMP1) is a viral protein that regulates certain human genes through the tumor necrosis factor-alpha/CD40 pathway [11]. Expression of this protein induces many of the changes that associated with EBV infection and activation of B cells [12]. Many studies had connected the infection with EBV to etiology of lymphoma in different parts of the world [13, 14, and 15], but such studies are virtually scanty in Sudan. Therefore, this study aimed to test the presence of EBV in lymphoma in Sudan using LMP 1 monoclonal antibody.

MATERIAL & METHODS:

This retrospective descriptive study was conducted during the period between July 2013 and January 2014 and included fifty paraffin-embedded formalin-fixed tissue blocks of lymphoma lesions. These tissue blocks were obtained from the archives of Radiation Isotope Center Khartoum (RICK) hospital and processed into Total Lab Care, Khartoum. Two 3-4 μ m-thick tissue sections were cut from each block using a rotary microtome. One section was put on a frosted-end glass slide and stained by Hematoxylin and Eosin stain for confirmation of diagnosis found in the records. The other section was

placed on positively charged slide and dried overnight at 58° C. Recommended Ventana®* Staining Procedure (ultraView™) using Ventana stainer machine was applied. All quality control measures were followed during specimen collection and processing. Statistical Package for Social sciences (SPSS) software version 20 was used for data entry and analysis. Results were tabulated and presented in percentage form. Parameters analyzed were age, sex, tumor type, and IHC results.

RESULTS:

About 62% of patients were above 40 years of age (table 1).

Table 1: Age distribution of cases in the study

Age group	Frequency	Percentage
Up to 10years	4	8 %
11 – 20 years	8	16 %
21-30 years	3	6 %
31-40 years	4	8 %
41-50 years	3	6 %
51-60years	11	22 %
61-70years	6	12 %
71-80 years	6	12 %
More than 80years	5	10%
Total	50	100%

NHL B cell type was the most frequent type in the studied cases (table 2).

Table 2: Frequency of different types of lymphoma in the study

Diagnosis	Frequency	Percentage
NHL Bcell type	36	72%
NHL Tcell type	2	4%
Hodgkin’s Lymphoma	12	24%
Total	50	100%

Most patients were males (72%); most of them (50%) were of NHL B cell type (table 3).

Table 3: Sex distribution within cases of the study

Lymphoma Type	Gender		Total
	Male	Female	
NHL B cell type	25/50 50%	11/50 22%	36/50 72%
NHL T cell type	2/50 4%	0 0%	2 4%
Hodgkin Lymphoma	9/50 18%	3/50 6%	12/50 24%
Total	36/50 72%	14/50 28%	50 100%

Only two cases were positive for EBV in the study, one was NHL B cell type and the other was Hodgkin’s Lymphoma type (table 4)

Table 4: Detection of EBV LMP 1 marker into Lymphoma lesions

Diagnosis	EBV		Total
	Positive	Negative	
NHL B type	1 2%	35 70%	36 72%
NHL T type	0 0%	2 4%	2 4%
Hodgkin lymphoma	1 2%	11 22%	12 24%
Total	2 4%	48 96%	50 100%

DISCUSSION:

Herpes virus family members include herpes simplex I and II and varicella-zoster virus (alpha virus subfamily), cytomegalovirus and human herpes virus 6 and 7 (beta herpes virus subfamily), and human herpes virus 8 and EBV (gamma herpes virus subfamily). [16]. Several Human tumors have been attributed to both human herpes virus 8 (Kaposi’s sarcoma, primary effusion lymphoma, and Castleman’s disease) and EBV (Burkitt’s lymphoma, nasopharyngeal carcinoma, and Hodgkin’s and non-Hodgkin’s lymphomas) [17].

Immunohistochemistry has developed as an efficient tool to demonstrate presence of EBV. Findings in this study are not unique; most of them are almost similar to several other studies in the field.

Ishtiaq S et al[18] studied 71 patients with NHL using EBV LMP-1 and reported that 12.7% of cases were positive for EBV. In this study, NHL cases were 38 and only one was positive for LMP 1 (3%). About 70.4% of patients were males; in this study it was 72%. B cell lymphomas were 86% and T cell lymphomas were 14%; in this study, about 95% of NHL cases were of B cell type and 5% of T cell type.

Al-Mudallal and Al-Sinjery[19] studied 50 paraffin-embedded blocks of Classical Hodgkin’s Lymphoma lesions and reported positive immunohistochemical expression of LMP-1 in 90% of the cases. Most of HD cases were in childhood and early adult age with male predominance. Our study agrees with male predominance and age of onset but disagree in percentage of positive cases.

AN Olu-Eddo and CE Omoti[20] studied 56 cases of Hodgkin lymphoma; most of them were males in the age groups 11-15 and 21-25.

Ashraf MJ et al[21] studied 30 cases of NHL B cell type and reported that all cases were negative for EBV LMP 1. The patients included 16 men and 14 women, with mean average age of 53.5 years (range from 9 to 81 years). The ratio of male to female was 8 to 7.

Audouin J et al[22] studied 55 cases of Hodgkin’s lymphoma and reported that 63% of them were positive for EBV LMP 1.

Chabay PA et al [23] performed Epstein-Barr encoded RNAs in situ hybridization and latent membrane protein 1 immunohistochemistry on formalin-fixed paraffin-embedded HL biopsies from 176 pediatric patients from two public institutions from Argentina and Brazil. EBV expression was detected in 52% of cases (54% Argentina and 48% Brazil). They concluded that the results did not support EBV association with HL in developing countries and, therefore, cannot be used as prognostic factor.

Karnik S et al [24] studied 100 HL cases to investigate the association of Epstein-Barr virus (EBV) latent membrane protein-1 (LMP-1) with HL in South India. EBV LMP-1 was positive in 82% of the cases.

CONCLUSION:

The only 4% positivity of EBV LMP 1 in lymphoma lesions is weak and does not support EBV association with lymphoma in our locality and, therefore, cannot be used as significant prognostic factor. To confirm, studies of larger numbers of patients are recommended.

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REFERENCES:

1. Cancer Facts & Figures 2014. Atlanta, GA: American Cancer Society; 2014.
2. Salwa HS, NHL in Soba University hospital 1979-1989, Master degree. 1992. Sudan. Soba University.
3. Intisar E. Saeed, Hsin-Yi Weng, Kamal H. Mohamed & Sulma I. Mohammed. Cancer incidence in Khartoum,

- Sudan: first results from the Cancer Registry, 2009–2010. *Cancer Medicine* 2014; 3(4): 1075–1084.
4. Jarrett RF. Viruses and lymphoma/leukemia. *J Pathol.* 2006; 208(2): 176-86.
 5. Hislop AD, Taylor GS, Sauce D, et al. Cellular responses to viral infection in humans: lessons from Epstein-Barr virus. *Annu Rev Immunol*, 2007; 25: 587-617.
 6. Jenkins, P. J. & Farrell, P. J. Are particular Epstein-Barr virus strains linked to disease? *Semin Cancer Biol*, 1996, 7: 209–215.
 7. Young, L. S., and P. G. Murray. Epstein-Barr virus and oncogenesis: from latent genes to tumors. *Oncogene*, 2003, 22:5108–5121.
 8. Andersson J. "Epstein–Barr virus and Hodgkin's lymphoma". *Herpes*, 2006, 13 (1): 12–6.
 9. Kapatai G and Murray P. Contribution of the Epstein–Barr virus to the molecular pathogenesis of Hodgkin lymphoma. *J Clin Pathol*, 2007; 60: 1342-9.
 10. Re D, Küppers R, Diehl V. Molecular pathogenesis of Hodgkin's lymphoma. *J Clin Oncol.* 2005; 23(26): 6379-86.
 11. Li, H; Chang, Y. "Epstein–Barr virus latent membrane protein 1: Structure and functions." *J Biomed. Sci.* 2003, 10: 490=504.
 12. Kaye, K. M., Izumi, K. M. & Kieff, E. Epstein-Barr virus latent membrane protein 1 is essential for B-lymphocyte growth transformation. *Proc Natl Acad Sci USA*, 1993, 90: 9150–9154.
 13. Chabay P, De Matteo E, Aversa Luis, et al. Assessment of Epstein-Barr virus association with pediatric non-Hodgkin lymphoma in immunocompetent and in Immunocompromised patients in Argentina. *Arch Pathol Lab Med*, 2002, 126, 331-5.
 14. Engert A, Ballova V, Haverkamp H, et al. Hodgkin's lymphoma in elderly patients: a comprehensive retrospective analysis from the German Hodgkin's Study Group. *J Clin Oncol.* 2005; 23(22): 5052-60.
 15. Huang X, Nolte I, GAO Z, et al. Epidemiology of classical Hodgkin lymphoma and its association with Epstein Barr virus in northern China. *PLoS One.* 2011; 6(6): e21152.
 16. Nourse JP, Crooks P, Keane C, et al. Expression profiling of Epstein-Barr virus-encoded microRNAs from paraffin-embedded formalin-fixed primary Epstein-Barr virus-positive B-cell lymphoma samples. *J Virol Methods*, 2012, 184, 46-54.
 17. Mushtaq S, Akhtar N, Jamal S, et al. Malignant Lymphomas in Pakistan According to WHO Classification of Lymphoid Neoplasms. *Asian Pac J Cancer Prev*, 2008, 9, 229-32.
 18. Ishtiaq S, Hassan U, Mushtaq S, Akhtar N. Determination of frequency of Epstein-Barr virus in non- Hodgkin lymphomas Using EBV latent membrane protein 1 (EBV-LMP1) immuno-histochemical staining. *Asian Pac J Cancer Prev.* 2013; 14(6):3963-7.
 19. Al-Mudallal and Al-Sinjery. Immunohistochemical Expression of Epstein Barr Virus Antigen Latent Membrane Protein-1 and Bcl-2 in Classical Hodgkin Lymphoma. *IRAQI J MED SCI*, 2012; VOL. 10(3).
 20. AN Olu-Eddo and CE Omoti. Hodgkin lymphoma: Clinicopathologic features in Benin City, Nigeria and update on its biology and classification. *Nigerian Journal of Clinical Practice*, 2011, 14 (4): 440-444.
 21. Ashraf MJ, Makarempour A, Monabati A, et al. Comparison between presence of EpsteinBarr virus in nodal and extra nodal diffuse large B cell lymphoma of head and neck, an Iranian experience. *Iran Red Crescent Med J*, 2012, 14: 764-70.
 22. Audouin J, Diebold J, Nathwani B, et al. Epstein–Barr virus and Hodgkin's lymphoma in Cairo, Egypt. *J Hematop.* 2010; 3(1): 11-8.
 23. Chabay PA, Barros MH, Hassan R, et al.: Pediatric Hodgkin lymphoma in 2 South American series: a distinctive epidemiologic pattern and lack of association of Epstein-Barr virus with clinical outcome. *J Pediatr Hematol Oncol*, 2008, 30 (4): 285-91.
 24. Karnik S, Srinivasan B, Nair S. Hodgkin's lymphoma: immunohistochemical features and its association with EBV LMP-1. Experience from a South Indian hospital. *Pathology.* 2003; 35: 207-11.