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Original Research Article

UTILITY OF PLASMA EPSTEIN-BARR VIRAL LOAD IN MANAGING HODGKIN'S LYMPHOMA: A PROSPECTIVE STUDY

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Abstract

Background: The study was aimed to measure the Epstein-Barr virus (EBV) association with Hodgkin's lymphoma (HL) and to determine the clinico-therapeutic role of plasma EBV quantification in EBV associated HL. **Materials and Methods:** This was a hospital based prospective study done on 40 HL patients. Plasma EBV DNA was quantified by using RT-PCR. Ann-Arbor staging system and RECIST criteria were applied to assess the severity of HL and therapeutic response, respectively. Data collected was analyzed statistically. **Result:** The mean age of the study group was 29.58±20.27 years ranging from 3 to 70 years. Plasma EBV DNA was detected in 42.5% study patients. The constitutional B symptoms were observed in 58.8% EBV positive(EBV+) and 26.1% EBV negative (EBV-)HL patients ($p<0.05$). Total 70.6% EBV+ patients and 30.43% EBV- patients had advanced stage disease (ASD) on presentation ($p = 0.01$). The prevalence of ASD among EBV+patients carrying high viral load (>800 copies/ml) and low advanced stage disease was 100% and 28.6%, respectively ($p = 0.001$). EBV DNA after three and six cycles of chemotherapy was undetectable in 58% and 94.1% EBV+ patients, respectively ($p<0.001$). **Conclusion:** EBV positive status had significant positive association with B symptoms and disease severity of HL. Serial quantification of circulating EBV DNA may help in monitoring disease severity; prognosis and response to therapy in EBV associated HL.

INTRODUCTION

Epstein Barr virus (EBV) is a ubiquitous tumor virus belonging to herpes virus family preferentially targeting human B cells.[1,2] It has been found to be associated with a variety of human malignancies such as Hodgkin lymphoma (HL), non-Hodgkin lymphomas (NHL), Burkitt's lymphoma, nasopharyngeal carcinoma and lymphomas associated with immunosuppression.[2,3] Approximately one-third cases of Classical HL that constitutes 95% of all HL cases are found to be associated with EBV infection, which is defined by the presence of EBV proteins or EBV-encoded RNA in tumor cells where its presence is thought to be causal.[4,5]

EBV-associated malignancies including HL are often diagnosed based on a biopsy of the primary tumor. However, because of the patient's poor health status or the difficulty in getting to the tumor, a biopsy can be challenging to perform sometimes. The measurement of EBV viral load in peripheral blood would be a less invasive and more practical method of EBV diagnosis. In fact, EBV viral load quantification has recently played a very important role in the diagnosis and management of EBV-associated diseases.[6,7]Additionally, it was discovered that individuals who responded to medication had significant reduction inbaseline EBV viral load,[8,9] highlighting the prognostic significance of EBV viral load.Hence, a serum or plasma EBV DNA detection assay has a good scope for being used as abiomarker in EBV associated malignancies including HL.

Data related to diagnostic and therapeutic role of circulating plasma EBV DNA levels among Indian HL patients are limited. The current study was related to detection of EBV in HL patients and to measure the association of EBV viral load with

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clinic-pathological parameters and therapeutic response in EBV associated HL patients.

MATERIALS AND METHODS

Study design: This was a hospital based prospective study conducted at a tertiary care institute of Northern India. The study was conducted onHL patients attending outpatient and indoor patient departments of internal medicine at our institute during January 2016 to March 2017. A total of forty newly diagnosed HL patients fulfilling the inclusion and exclusion criteria were included as subject in the study. The diagnosis of HL was confirmed by proper histopathological examination and immuno-histo-chemical (IHC) analysis of biopsied lymph node sample from clinically suspected HL patients.

HL Patients with following conditions were excluded from the study group:

Patients who were not willing to give informed consent.

HIV positive patients having HL

HL patients already on chemotherapy

Relapse cases

Patient with concomitant other hematological malignancy and Solid tumors.

Patients already receiving other chemotherapy for other illnesses.

Each enrolled subject was evaluated for disease Severity as assessed by Ann Arbor staging system.[10] Staging evaluation included acomplete history, thorough physical examination, imaging(computed tomography scan of the thorax, abdomen,and pelvis), and bone marrow aspiration and biopsy.[11] Positron emission tomography (PET) scan was not done on any patient due to its non-availability at our center and poor patient affordability for getting it done from outside.

Each subject was then assessed for EBV status for which 5ml of peripheral blood sample was collected and processed for extraction of DNA by automated extraction system followed byquantification of EBV DNA by real time polymerase chain reaction (RT PCR). Titers were interpreted as follows: undetectable = EBV negative; detectable= EBV positive; low viral load = ≤800 copies/ml; high viral load= >800 copies/ml. Plasma EBV viral load was re-quantified for each EBV positive patient after 3rd and 6th cycle of chemotherapy (C3 and C6, respectively). No repeat EBV viral load testing was done for patients who came negative during baseline EBV DNA testing.

All patients with EBV positive HL (EPHL) and EBV negative HL (ENHL) received six cycles of Adriamycin, Bleomycin, Vinblastine and Dacarbazine based chemotherapy (i.e. ABVD regimen) throughout the course of study with an inter-cycle period of at least a month.

Therapeutic response (TR) was assessed for all patients after C3 and C6 by applying Response Evaluation Criteria in Solid Tumors (RECIST criteria) and was comparatively interpreted in relation to their EBV status.[12]

Ethical Approval: Ethical clearance for the study was obtained from the ethics committee of the institute. A written, informed consent was obtained from all patients enrolled in the study.

Statistical Analysis: The acquired data was statistically analyzed using Statistical Package for Social Sciences (version 20; SPSS, Chicago, IL, USA). Categorical variables were presented as number and percentages, and quantitative variables were presented as mean and standard deviation (SD). Chi-square test was applied to ascertain association between categorical variables. The significance threshold was set at a p-value of less than 0.05.

RESULTS

A total of forty patients with HL (30 male and 10 female) were evaluated and followed over a period of six months in this study. Mean age of the study group was 29.58±20.27 years with a range between 3 to 70 years [Table 1]. Most patients (i.e. 70%) belonged to age group of less than 20 years [Table 1]. Plasma EBV DNA was detected in 17 (42.5%) patients of the study group. Among 17 EBV positive patients 8 (47%) patients belonged to age of less than 20 years. however, we didn't find any significant EBV association with age and gender of the patients [Table 1].

The most common histological subtype in EPHL group was mixed cellularity HL (MCHL) whereas it was nodular sclerosis HL (NSHL) in ENHL group (p value <0.05) [Table 1].

Total 40% patients from the study group had constitutional B symptoms on presentation. In relation to EBV status, 58.8% patients from EPHL group and 26.1% patients from ENHL group had constitutional B symptoms. This difference was found to be statistically significant with p value of 0.036 [Table 2].

When study population was assessed for disease severity as per Ann Arbor staging in relation to their EBV status, it was found that majority of the patients (12 out of 17 i.e. 70.6%) from EPHL group had advanced stage disease (IIB, III & IV) in comparison to ENHL group in which only 20.4% (7 out of 23) patients had advanced stage disease. This difference was found to be statistically significant with p value of 0.01 [Table 3].

As far as EBV viral load is concerned it was observed that all EPHL cases with high viral load (>800 copies/ml) were found to have advanced stage of the disease whereas most EPHL cases with low viral load (<800 copies/ml) had milder form of the disease (p= 0.001) [Table 4].

A statistically significant drop in viral load on follow up RT PCR was noted in most EPHL patients. EBV viral load was reduced to

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undetectable level in 58% patents post C3 and in 94.1% patients post C6 (p<0.001) [Table 5].

However, on comparing therapeutic responses in EPHL and ENHL groups, no significant difference was found on post C3 as well as post C6 assessment (p value = 0.093 and 0.866, respectively) [Table 6].

Table 1: Baseline characteristics of the study group according to their EBV status.

Characteristics		Total(n = 40)	EBV + ve(n = 17)	EBV –ve(n = 23)	$\chi 2$	P value
Age (Years)	0-19	18	8	10	0.943	0.815 (NS)
	20-59	20	8	12		
	≥60	2	1	1		
Mean Age(Years)	Study group	29.58	31.24	28.35		
Gender	Male	30	13	17	0.034	0.853 (NS)
	Female	10	4	6		
HL Subtypes	MC	18	13	5	12.947	0.012 (S)
	NSHL	18	3	15		
	LRHL	2	1	1		
	LDHL	1	0	1		
	NLPHL	1	0	1		

EBV= Epstein Barr Virus; +ve= positive; -ve= negative; $\chi 2$ = chi square statistic;NS = non-significant; S = significant; HL= Hodgkin lymphoma; MCHL= mixed cellularity HL; NSHL= nodular sclerosis HL; LDHL= lymphocyte-depleted HL; LRHL= Lymphocyte rich HL; NLPHL= nodular lymphocyte-predominant HL.

Table 2:Relation of EBV status with constitutional B symptoms among HL patients.

B symptoms	Total (n=40)	EBV status		P value
		EBV + ve(n = 17)	EBV –ve(n = 23)	
Present	16 (40%)	10 (58.8%)	6 (26.1%)	0.036 (S)
Absent	24 (60%)	7 (41.2%)	17 (73.9%)	

EBV= Epstein Barr Virus; B symptoms = unexplained profound weight loss, high fevers, and drenching night sweats; HL= Hodgkin lymphoma;+ve= positive; -ve= negative; S = significant.

Table 3: Relation of EBV status with disease severity among HL patients.

Disease severity	Ann Arbor staging	EBV status		P value
		EBV + ve(n = 17)	EBV –ve(n = 23)	
Early stage	(I, IIA)	5 (29.4%)	16 (69.6)	0.01 (S)
Advanced stage	(IIB, III, IV)	12 (70.6%)	7 (30.4%)	

HL= Hodgkin lymphoma; EBV= Epstein Barr Virus; +ve= positive; -ve= negative; S = significant

Table 4: Relation of baseline EBV Viral load with disease severity among EBV+HL (EPHL) patients.

Disease severity	Ann Arbor staging	Viral load (copies/ml)		P value
		≤800 (n= 7)	>800 (n= 10)	
Early stage	(I, IIA)	5 (71.4%)	0 (0%)	0.001 (S)
Advanced stage	(IIB, III, IV)	2 (28.6%)	10 (100%)	

Table 5: Change in EBV viral load after receiving treatmentamong EBV positive HL patients.

Viral load (copies/ml)	Pre-chemotherapy (at Baseline)	Post-chemotherapy	
		Post 3rd cycle	Post 6th cycle
Undetectable	0	10 (58.8%)	16 (94.1%)
Low (≤800)	7 (41.2%)	6 (35.3%)	1 (5.9%)
High (>800)	10 (58.8%)	1 (5.9%)	0 (0%)
P value when compared with baseline value(Chi square test)		<0.001 (S)	<0.001 (S)

Table 6: Comparative analysis of therapeutic response after 3rd and 6th cycle of chemotherapy in EPHL and ENHL patients

Timing of assessment	Response	Total (n=40)	EBV + ve(n=17)	EBV –ve(n=23)	p value
Post 3rd cycle	Complete	26 (65%)	8 (47.05%)	18 (78.3%)	0.093 (NS)
	Partial	13 (32.5%)	8 (47.05%)	5 (21.7%)	
	SD	1 (2.5%)	1 (5.9%)	0 (0%)	
	PD	0 (0%)	0 (0%)	0 (0%)	
Post 6th cycle	Complete	31 (77.5%)	12 (70.6%)	19 (82.6%)	0.866 (NS)
	Partial	1 (2.5%)	1 (5.9%)	0 (0%)	
	SD	2 (5%)	1 (5.9%)	1 (4.3%)	
	PD	6 (15%)	3 (17.6%)	3 (13.1%)	

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EBV= Epstein Barr Virus; +ve= positive; -ve= negative; EPHL= EBV positive HL; ENHL= EBV negative HL; HL= Hodgkin lymphoma;SD= stable disease; PD= progressive disease; NS = non-significant

DISCUSSION

Hodgkin lymphoma is a well-established malignancy having many riskfactors such as age, gender, autoimmune diseases, immunosuppression, family history, alcohol consumption, smoking habits, and EBV infection.[11] EBV has been found to play a major contributing role in its pathogenesis.[1]However, the exact etiology of HL is still unknown. In overcrowded living and unsanitary conditions of developing countries, primary EBV infection occursat a much earlier age so thatby the age of two, 90% of children are seropositive.[13] This is partly responsible forgreater EBV associated HL in developing countries like India than in developedcountries.[13-15] Several studies have proven the role of EBV viral loads by quantitative PCR in assessing disease association, risk stratification, and therapeutic response in HL patients.[6, 14-18]

The demographic profile of our study group was moderately similar to that of previous studies.[19,20] In this study,40% of the patients had constitutional ‘‘B” symptoms that was similar to the study done by Sinha M et al in which 39% study subjects had constitutional ‘‘B” symptoms.[19] Similarly Hohaus S et al reported 35% HL study patients having B symptoms.[16] In developed countries, ‘‘B” symptoms are found in 25–30% of HL, whereas in developingcountries it is found in about 50% of cases.[9,13] An even higher prevalence of B symptoms has been reported in Indian children with HL.[21,22]Dinand V et al,[21] and Arya et al,[22] have reported B symptoms in 52.6% and 54.4% children with HL, respectively.

EBV positivity in HL varies with geographical location; 20–50% in the West, 57–64% in the Far East, and up to 90% in developing countries.[3,18,23,24]Dinand V et al found EBV positivity in 96.6% of HL patients that was significantly associated with younger age (p=0.012) and lower socioeconomic level (p=0.007).[9] In another study done on childhood HL, 63% children were found positive for EBV.[21] Using plasma EBV DNA estimation in adult-onset HL, an Indian study revealed a 48.5% EBV association.[19] A Brazilian study also reported a 43% EBV association in adult HL population.[20] These studies showed that EBV positivity is relatively high in childhood HL patients in comparison to adult HL patients. In present study we found EBV association in 42.5% HL patients. The reason for lower prevalence in our study might be higher mean age of the study group in comparison to previous studies,[9,21] which was mainly done on childhood HL.

MCHL and NSHL are common subtypes in Indian HL patients as reported in previous studies.[21,22]Dinand V et al and Arya et al, observed MCHL in 63% and 86% study subjects, respectively,[21,22] while Sinha M et al and Rani P et al reported NSHL in 48.5% and 79.5% study population, respectively.[19,25] However, we observed MCHL and NSHL in equal number (i.e. 45% each) of our study subjects. As far as EBV is a concern, MCHL was the commonest subtype in EPHL group in the present study [Table 1], which was similar to the previous studies.[21,23,26-28]

In the present study we observed relatively higher prevalence of advanced stage disease among EPHL patients in comparison to ENHL patients [Table 3].

Similarly as per review analysis by NohtaniMet al it was concluded that an EBV-positive status is associated with poorer clinical outcomes especially in elderly patients.[29]

A positive association between baseline EBV viral load and disease severity in EBV associated HL patients has been reported in several studies.[16,25,30,31]Dinand V et al demonstrated a higher prevalence of advanced stage disease (IIB, III, IV) among EPHL patients carrying high viral load in comparison to those carrying relatively a low viral load in their study.[21] As per study done by Hohaus S et al,[16] high EBV DNA in plasma of HL patients corresponds tohigher stage of the disease in HL patients, which support our study findings in this context [Table 4].

EBV viral load have been studied for monitoring therapeutic response in EBV positive lymphomas in several studies.[8,9,14,15,17,19,32] Gandhi MK et al,[14] and Spacek M et al,[17] have conclusively found therapeutic response to be associated with decline in EBV viral load in their studies. Clearance of EBV DNA after chemotherapy was found to be associated with remission in all HL patients in a study done by Sinha M et al.[19] Similarly, a statistically significant drop in viral load with treatment was noted in the present study [Table 5]. Thus our findings as well as previous research are in favor of prognostic importance of EBV viral load in managing EBV associated HL patients.

This study had certain limitations. The study was conducted in a singlecenter with a relatively limited samplesize. The follow up period in the present study was relatively shorter then the previous studies hence, long term effect of EBV in HL patients could not be established. We could not be able to know the exact burden of EBV among general population due to lack of control group in our study, hence, could not be compared with that of our study group. The authors further recommend larger cohort studies with longer follow up period to confirm and validate our study findings.

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CONCLUSION

EBV infection had strong positive association with disease severity and constitutional B symptoms. Change in EBV viral load after treatment was found to be concordant with therapeutic response in EBV associated HL. Hence, it could be used as a noninvasive biomarker to monitor therapeutic response in EBV associated HL, which may help in deciding the further plan of therapy.

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REFERENCES

1. Cohen JI. Epstein Barr Virus Infection including Infectious Mononucleosis. In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J, editors. Harrison's Principles Internal medicine 19th edi new york: Mcgraw hill, 2015;pp.1186-90.
2. Rickinson A. Epstein–Barr virus. Virus Res. 2002 Jan 30;82(1-2):109–13.
3. Hsu JL, Glaser SL. Epstein–Barr virus-associated malignancies: epidemiologic patterns and etiologic implications. Crit Rev Oncol Hematol2000;34:27–53.
4. Jarrett RF. Risk Factors for Hodgkin's Lymphoma by EBV Status and Significance of Detection of EBV Genomes in Serum of Patients with EBV-Associated Hodgkin's Lymphoma. Leukemia& Lymphoma 2003;44(sup3):S27-S32.
5. Kaseb H, Babiker HM. Hodgkin Lymphoma. [Updated 2022 Jul 10]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-.
6. Kimura H, Ito Y, Suzuki R, Nishiyama Y. Measuring Epstein-Barr virus (EBV) load: the significance and application for each EBV-associated disease. Rev Med Virol. 2008;18:305–19.
7. Kanakry J, Ambinder R. The biology and clinical utility of EBV monitoring in blood. Curr Top Microbiol Immunol. 2015;391:475–99.
8. artus EBV QS-RGQ Kit Handbook. March 2015 p1-p58.
9. 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 Jan;43(1):161-8.
10. Lister TA, Crowther D, Sutcliffe SB, Glatstein E, Canellos GP, Young RC, Rosenberg SA, Coltman CA, Tubiana M. Report of a committee convened to discuss the evaluation and staging of patients with Hodgkin's disease: Cotswolds meeting. J Clin Oncol. 1989 Nov;7(11):1630-6.
11. Longo DL. Malignancies of Lymphoid cells. In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J, editors. Harrison's Principles Internal medicine 19thedi new york: Mcgraw hill, 2015;pp.695-709.
12. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst. 2000 Feb 2;92(3):205-16.
13. Naresh KN, Johnson J, Srinivas V, Soman CS, Saikia T, Advani SH, et al. Epstein–Barr virus association in classical Hodgkin's disease provides survival advantage to patients and correlates with higher expression of proliferation markers in Reed–Sternberg cells. Ann Oncol 2000;11:91–6.
14. Gandhi MK, Lambley E, Burrows J, Dua U, Elliott S, Shaw PJ, et al. Plasma Epstein–Barr virus (EBV) DNA is a biomarker for EBV-positive Hodgkin lymphoma. Clin Cancer Res 2006;12:460–4.
15. Kanakry JA, Li H, Gellert LL, Lemas MV, Hsieh WS, Hong F, et al. Plasma Epstein–Barr virus DNA predicts outcome in advanced Hodgkin's lymphoma: correlative analysis from a large North American co-operative group trial. Blood 2013;121:3547–53.
16. Hohaus S, Santangelo R, Giachelia M, Vannata B, Massini G, Cuccaro A, et al. The viral load of Epstein–Barr virus (EBV) DNA in peripheral blood predicts for biological and clinical characteristics in Hodgkin lymphoma. Clin Cancer Res 2011;17:2885–92.
17. Spacek M, Hubacek P, Markova J, Zajac M, Vernerova Z, Kamaradova K, et al. Plasma EBV-DNA monitoring in Epstein Barr virus-positive Hodgkin lymphoma patients. Acta PatholMicrobiol Immunol Scand 2010;119:10–6.
18. Flavell KJ, Murray PG. Hodgkin's disease and the Epstein–Barr virus. J Clin Pathol Mol Pathol2000;53:262–9.
19. Sinha M, Rao CR, Shafiulla M, Shankaranand B, Viveka BK, Lakshmaiah KC, Jacob LA, Babu GK, Jayshree RS. Plasma Epstein Barr viral load in adult-onset Hodgkin lymphoma in South India. Hematol Oncol Stem Cell Ther. 2016 Mar;9(1):8-13.
20. Musacchio JG, Carvalho Mda G, Morais JC, Silva NH, Scheliga A, Romano S, et al. Detection of free circulating Epstein-Barr virus DNA in plasma of patients with Hodgkin's disease. Sao Paulo Med J. 2006 May 4;124(3):154-7.
21. Dinand V, Sachdeva A, Datta S, Bhalla S, Kalra M, Wattal C, et al.Plasma Epstein-Barr Virus (EBV) DNA as a Biomarker for EBV- associated Hodgkin lymphoma. Indian Pediatr 2015;52: 681-5.
22. Arya LS, Dinand V, Thavaraj V, Bakhshi S, Dawar R, Rath GK, et al. Hodgkin's disease in Indian children: outcome with chemotherapy alone. Pediatr Blood Cancer. 2006 Jan;46(1):26-34.
23. 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 Feb 7;70(4):375-82.
24. Ambinder RF. Epstein-barr virus and hodgkin lymphoma. Hematology Am Soc HematolEduc Program. 2007:204-9.
25. Rani P, Jain M, Verma N, Kumar A, Jain A, Tripathi AK, et al. Epstein–Barr Virus Expression in Classic Hodgkin Lymphoma in an Indian Cohort and its Association with Clinical and Histomorphological Parameters. Indian J Hematol Blood Transfus. 2021;37(3):372–8.
26. Jarrett AF, Armstrong AA, Alexander E. Epidemiology of EBV and Hodgkin's lymphoma. Ann Oncol 1996;7(suppl 4):S5–10.
27. Pallesen G, Hamilton-Dutoit SJ, Rowe M, Young LS. Expression of Epstein-Barr virus latent gene products in tumour cells of Hodgkin's disease. Lancet. 1991 Feb 9;337(8737):320-2.
28. 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 Jan;166(1):1–5.
29. Nohtani M, Vrzalikova K, Ibrahim M, Powell JE, Fennell É, Morgan S, et al. Impact of Tumour Epstein–Barr Virus Status on Clinical Outcome in Patients with Classical Hodgkin Lymphoma (cHL): A Review of the Literature and Analysis of a Clinical Trial Cohort of Children with cHL. Cancers 2022;14:4297.
30. Gallagher A, Armstrong AA, MacKenzie J, Shield L, Khan G, Lake A, et al. Detection of Epstein-Barr virus (EBV) genomes in the serum of patients with EBV-associated Hodgkin's disease. Int J Cancer. 1999 Aug 20;84(4):442-8.
31. Kimura H, Morita M, Yabuta Y, Kuzushima K, Kato K, Kojima S, et al. Quantitative analysis of Epstein-Barr virus load by using a real-time PCR assay. J Clin Microbiol. 1999 Jan;37(1):132-6.
32. Au WY, Pang A, Choy C, Chim C, Kwong Y. Quantification of circulating Epstein–Barr virus (EBV) DNA in the diagnosis and monitoring of natural killer cell and EBV-positive lymphomas in immunocompetent patients. Blood 2004;104:243–9.

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