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Original Article

A Meta-Analysis of Potential Relationship between Epstein-Barr-Encoded-RNA (EBER) and Onset Time of Post-Transplant Lymphoproliferative Disorders

Hossein Khedmat[1], Reza Karbasi-Afshar[2], Shahram Agah[3], Mohammad Ebrahim Ghamar-Chehreh[1], Mohsen Amini[1]

[1]Baqiyatallah Research Center for Gastroenterology and Liver Disease, [2]Atherosclerosis Research Center, Baqiyatallah University of Medical Sciences, [3]Colorectal Research Center, Iran University of Medical Sciences, Tehran, Iran

ABSTRACT. Epstein-Barr virus (EBV) encodes two non-polyadenylated RNAs termed EBV-encoded RNAs (EBERs). In this study, we tried to find series in which data of EBER and onset time of post-transplant lymphoproliferative disorder (PTLD) for patients have been documented to conduct a meta-analysis. A comprehensive search of the literature was performed by Pubmed and Google scholar to find reports indicating test results for EBER and PTLD onset in transplant patients. PTLD was considered "early onset" when it develops within the first post-transplant year. Finally, 265 patients from 15 studies have been included in the meta-analysis. The overall meta-analysis also showed a significant relation between EBER test positivity and early-onset PTLD development [relative risk (RR): 1.36; 95% CI: 1.16–1.59; *P* <0.001]. The i2 index was 49.8%. Our study suggests that PTLD lesions with positive EBER test are more likely to develop within the early post-transplant period. Since early-onset PTLD is supposed to have better prognosis, having a positive EBER test might not be a bad news. However, for having a precise conclusion, prospective studies are needed to be conducted.

Introduction

Post-transplant lymphoproliferative disorder (PTLD) is usually referred to a wide spectrum of abnormal lymphatic proliferations, which have become clinically more relevant, especially in the recent two decades due to the substantial advancements in transplantation medicine and development of highly potent immunosuppressive agents for preventing or treating rejection episodes.[1] Besides the immunosuppression employed, the Epstein-Barr virus (EBV) has also been shown to have a major causative effect on the development of PTLD as it has been detected in up to 90% of PTLD lesion cells.[2,3]

EBV is an opportunistic pathogen that subs-

Correspondence to:

Dr. Reza Karbasi-Afshar
Atherosclerosis Research Center,
Baqiyatallah University of Medical Sciences,
Tehran, Iran
E-mail: karbasi.afshar@gmail.com

tantially affects the prognosis of immunocompromised patients.[4] *In vitro*, EBV infects resting B cells and transforms them into proliferating blasts, which results in unregulated polyclonal expansion of latently infected lymphoblasts.[5,6] In the absence of an appropriate EBV-specific cytotoxic T-cell response, probably caused by the administration of immunosuppression after transplantation, the transformed cells increase the risk of neoplasm development in these patients. PTLD is one of the malignancies shown by overwhelming evidence to be related with EBV infection. Because transplant recipients usually receive aggressive immunosuppressive medication to prevent rejection episodes and graft loss, they are at substantial risk for PTLD.

EBV encodes two non-polyadenylated RNAs termed EBV-encoded RNAs (EBERs). EBER1 and EBER2 are the most abundant viral transcripts in EBV-infected cells.[7,8] EBERs have recently received higher attention because newer evidence suggests the possibility that they could be involved in oncogenesis. In the transplant era, due to the overall limited number of PTLD occurrence in single centers, there is an extreme level of data scarcity on the subject. In this study, we garnered available series in which data of EBER and onset time of PTLD for patients have been documented and conducted a meta-analysis.

Materials and Methods

Search strategy

A comprehensive search of the literature was performed by Pubmed and Google scholar to find reports indicating test results for EBER and PTLD onset in transplant patients. PTLD was considered early onset when it started within the first year post-transplantation. Keywords used for this purpose included: PTLD, early onset, late onset, EBER, EBV encoded RNA or a combination of them. Because the found studies were not originally designed as case–control studies, we extracted data of patients of each study and included patients with a positive result for EBER in their PTLD lesions as the case group and those with a negative one as the controls. Only the studies with patients in both groups were included. From 28 studies, finally, data from 15 published studies[9-23] were included in the meta-analysis. Overall, 265 transplant recipients representing PTLD were included in the meta-analysis. Table 1 summarizes data used from each study with regard to the study groups.

Statistical Analyses

Softwares used for data analyses were SPSS v. 13.0 (SPSS CORP., Chicago, IL, USA) and Stata v. 9.0 (Stata Corp, Texas, TX, USA). All

Table 1. characteristics of the included studies.

Study ID	Study	Population	Age (year)	Gender male (%)	Early onset (%)	EBER positive (%)
1	Duvoux et al[9]	13	55 ± 6	9 (70)	11 (85)	9 (69)
2	Lucioni et al[10]	17	42 ± 15	16 (94)	3 (18)	9 (53)
3	Muti et al[11]	40	51 ± 13	32 (82)	7 (18)	27 (69)
4	Vakiani et al[12]	18	28 ± 22	10 (56)	6 (33)	11 (61)
5	Capello et al[13]	14	49 ± 8	12 (86)	9 (64)	10 (71)
6	Djokic et al[14]	25	26 ± 21	19 (76)	11 (44)	18 (72)
7	Buadi et al[15]	15	53 ± 15	9 (60)	11 (73)	14 (93)
8	Collins et al[16]	22	7 ± 5	-	13 (59)	20 (91)
9	Vilchez et al[17]	9	45 ± 17	6 (75)	5 (56)	6 (67)
10	Abe et al[18]	5	36 ± 10	3 (60)	1 (20)	2 (40)
11	Sun et al[19]	4	-	-	1 (25)	3 (75)
12	Berg et al[20]	28	35 ± 21	17 (61)	12 (43)	26 (93)
13	Chen et al[21]	42	14 ± 14	22 (53)	3 (7)	37 (90)
14	Johnson et al[22]	2	45 ± 18	1 (50)	1 (50)	1 (50)
15	Ifthikharuddin et al[23]	11	36 ± 21	6 (55)	9 (82)	10 (91)

statistical tests were performed at the 0.05 significance level.

Results

Patients

Data of overall 265 transplant recipients developing lymphoproliferative disorders after transplantation were entered into the analysis. There were 162 (69%) male and 74 (31%) female patients (29 unreported). Mean age at diagnosis of PTLD was 35 ± 22 years. The mean interval between transplantation and the diagnosis of PTLD was 50 ± 50 months, whereas the follow-up time after diagnosis of PTLD was 25± 32 months. Characteristics of the patients of each included study have been summarized in Table 1.

Meta-analysis

In the analysis, none of the included studies showed a significant positive association between having a positive EBER test and having a late-onset PTLD. Although most of the studies had a tendency toward a higher rate of EBER positivity and early-onset PTLD, in 13 of them, the 95% confidence interval (CI) crossed the neutral (1) line and significance level was only achieved in two of the included reports (IDs 2 and 6); also, the overall meta-analysis also showed a significant relation between EBER test positivity and early-onset PTLD development [relative risk (RR): 1.36; 95% CI: 1.16–1.59; P <0.001)] (Figure 1). The *i2* index was 49.8%, suggestive of a moderate to high heterogeneity. When the analysis has been repeated by removing single studies from the pooled data, the outcome saved its significance level, suggestive of no major effect from a single large study to put a significant deviation on the overall pooled analysis.

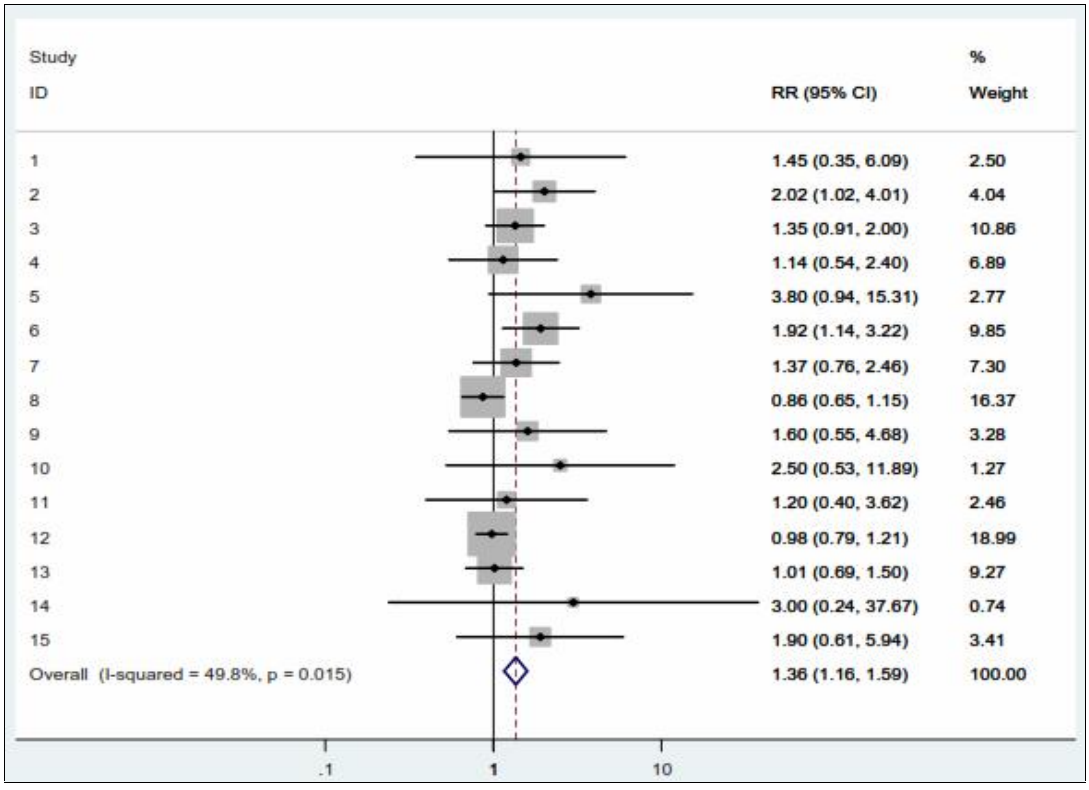

Figure 1. Meta-analysis of post-transplant lymphoproliferative disorder patients with a positive Epstein-Barr-encoded RNA test result; the Forest plot of the meta-analysis that includes all the participants from the 15 included reports.

Discussion

Infectious diseases are among the key players that adversely affect the lives of people of different subpopulations all around the world, but their significant role would become more prominent in patients with impaired immune systems. In the context of transplant, patients are extremely vulnerable to the effects of infectious agents, and infection is one of the major factors producing morbidity and mortality in this patient population.[24,25] EBV is one of the most investigated infections in transplant patients, which is supposed to have a causative role in their post-transplant period.[26] Moreover, it has been suggested that this infection can affect mortality of transplant patients developing PTLD.[27] On the other hand, it has been suggested that EBV-positive patients are significantly more likely to develop early-onset PTLD (than late onset) compared with EBV-negative transplant recipients.[28]

EBERs are the most common viral transcripts found in EBV-infected cells and have been found within the PTLD lesions of several patients in different series.[29] EBER positivity result is also an important issue in conducting therapeutic interventions such as changes in immunosuppression, rituximab therapy and chemotherapy.[30] In pediatric organ transplant patients, EBER positivity has been associated with immunohistochemical changes in tonsils.[31] In a previous review article, we showed that EBER positivity was able to predict the histopathology and prognosis of PTLD in transplant recipients.[32] In the current study, we performed a meta-analysis of 15 studies in which the authors have reported PTLD cases with positive and negative EBER tests to identify whether this test can predict the neoplasm onset time.

This study showed that EBER-positive PTLD lesions are significantly more likely to develop in transplant recipients who represent the disease within the first post-transplant year. However, our findings should be considered with caution as the heterogeneity in our meta-analysis was not limited (about 50%) and also because of the overall limited number of the included subjects. Moreover, data of the patients from the included studies were not derived from prospective case–control studies.

In conclusion, our study suggests that PTLD lesions with positive a EBER test are more likely to develop within the early post-transplant period. Because early-onset PTLDs are supposed to have better prognosis, having a positive EBER test might not be bad news. For having a precise conclusion, prospective studies have to be conducted.

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