Published in final edited form as:
Pediatr Transplant. 2010 June ; 14(4): 549–553. doi:10.1111/j.1399-3046.2009.01283.x.

Chronic High Epstein-Barr Viral Load Carriage in Pediatric Small Bowel Transplant Recipients

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Abstract

The development of Epstein-Barr virus (EBV) infection and post-transplant lymphoproliferative disorder (PTLD) is normally associated with a high EBV load in peripheral blood. Often, children undergoing primary or reactivation of EBV infection subsequent to intestinal transplantation (ITx) will have chronically elevated EBV loads. To better understand this phenomenon and its consequences, we retrospectively reviewed the records of children who underwent ITx (either isolated or part of multivisceral transplantation) at our center from 1992–2007 to identify chronic high EBV load carriers in this population. Chronic high load (CHL) state was defined as the presence of high load for >50% of samples for ≥6 months following either asymptomatic infection or complete clinical resolution of EBV disease/PTLD. 35 CHL carriers were identified from our patient population. Pre-transplant serologies were available on 34 of these patients: 17 were EBV negative and 17 seropositive; one had unknown EBV serostatus prior to transplant. 7 of the 17 seronegative patients developed their CHL carrier state at the time of their primary EBV infection. 13 of the 35 (35%) HLC patients developed EBV disease after meeting the definition of high load carrier states. EBV related diseases developing in CHL carriers included: EBV adenitis (n=1), EBV enteritis (n=7), PTLD (n=4) and EBV+ spindle cell tumor (n=1). Disease was seen in 7/17 of the seronegative (1 PTLD) and 6/17 of the seropositive patients (3 PTLD). 13/35 (37%) resolved their CHL state without apparent sequelae while 9 remain asymptomatic CHL carriers. Three children have had more than one episode of CHL. These data provide important information about the outcome of chronic EBV high load carriage in pediatric intestinal transplant recipients.

Introduction

Epstein - Barr virus (EBV) is an important cause of disease in pediatric transplant recipients which is responsible for significant morbidity and mortality (1–3). Disease manifestations vary from non-specific febrile syndrome to the more ominous post-transplant lymphoproliferative disorders (PTLD). The importance of EBV disease and PTLD has led to the development of enhanced tools to improve our ability to recognize and diagnose infection due to this viral pathogen. The use of polymerase chain reaction (PCR) detection of EBV load in peripheral blood has greatly improved our ability to diagnose and monitor patients with EBV disease (4–7). Further, surveillance of EBV loads allows for more rapid detection of EBV disease, and thus more prompt treatment of disease. Being able to use viral load measurement for detection of disease is especially important as EBV-associated PTLD

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in children is usually preceded by a very high rise in viral load before the onset of clinical symptoms. However, it is important to note that not all patients developing an elevated EBV load will progress to clinical disease. Elevated loads can be observed in pediatric transplant recipients experiencing asymptomatic infection or symptomatic EBV disease not meeting the criteria for PTLD.

As longitudinal experience with EBV monitoring accrued, a new phenomenon was observed, particularly in pediatric transplant recipients. Although in the typical course of EBV infection there is a sharp rise in viral load which gradually declines with the resolution of infection, we have observed that in a subset of transplant patients viral loads rise and then remain chronically high in the absence of clinical symptoms (8–12). In our experience, these elevated viral loads can remain persistently high for years after infection. We have previously defined patients with persistently elevated EBV loads as chronic high load (CHL) carriers if they meet the following criteria: high viral load for greater than 50% of samples for more than six months following either asymptomatic infection or complete resolution of EBV disease/PTLD (10). The long term clinical significance of an asymptomatic high load carrier state is still relatively unknown (11). However, a recent retrospective study of pediatric heart transplant patients found that 45% of these CHL patients progressed to late onset PTLD (8) while in pediatric liver transplant patients only 2.7% of patients progressed to PTLD (10). Thus, the type of solid organ transplanted and the differing post-transplant immunosuppressive management used in these CHL patients may lead to differential outcome. The purpose of the current study was to retrospectively review our experience at the Children's Hospital of Pittsburgh to characterize the outcome of the chronic high EBV load carrier state in pediatric intestinal transplant recipients.

Methods and Patient Population

EBV load monitoring

EBV load monitoring for intestinal transplant recipients was initiated in 1994 on a research basis for the purposes of instituting preemptive therapy for PTLD. By 1997, EBV load monitoring became routinely available for clinical use at Children's Hospital of Pittsburgh. Prior to 2002, EBV load was measured by quantitative competitive PCR on mononuclear cells measured as copies/105 peripheral blood mononuclear cells (PBMC) (5). However, beginning in October 2002, real time PCR on whole blood for viral load quantification was instituted and expressed as copies/mL of whole blood. The correlation between the two assays has been previously described (13).

Patient Population and Surveillance Frequency

All children who underwent intestinal transplantation, including multivisceral transplantation, at Children's Hospital of Pittsburgh and who had greater than or equal to five serial EBV load measurements between 1994 and 2007 were eligible for the study. The study was approved by the University of Pittsburgh Institutional Review Board. Beginning in 1994, prospective surveillance was performed beginning from the time of intestinal transplantation for all ITx recipients regardless of pre-transplant donor or recipient EBV serologic status. Surveillance was carried out according to the following schedule: every two weeks for the first three months, then monthly for the next three months, followed by every other month in the last 6 months of the first year out of transplant. After the first year out of transplant, patients were monitored for levels every three to four months. In the event of rising or persistent elevation of viral load or evidence of EBV disease, increased frequency of testing was clinically indicated.

Definition of Chronic High Load Carrier State

A chronic high load carrier is defined by carrying high viral loads for greater than 50% of samples for a greater than 6 month period following either asymptomatic infection or complete resolution of initial presentation with EBV disease/PTLD. High viral loads were defined as greater than 200 genome copies/10^5 PBL (prior to 2002) or greater than 16,000 genome copies/ml whole blood (after 2002).

Disease states

EBV disease was diagnosed when a clinical illness consistent with EBV infection in a transplant patient (and supported by serological and PCR evidence of EBV infection) occurred in the absence of extra-nodal mass lesions or histological evidence of PTLD (1). Examples included patients with fever, rash, hepatitis, enteritis, adenopathy or tonsillar hypertrophy in association with primary EBV seroconversion, IgM reactivation, or development of high viral load (> 16,000 copies / ml whole blood or > 200 copies /10^5 PBMC). PTLD was diagnosed histologically in all cases and was classified according to WHO criteria (14). Of note, in our center, ‘early lesions’ within lymphoid tissues without tissue destruction are not labeled as cases of PTLD.

Results

A total of 166 children underwent intestinal transplantation (including isolated intestine, combined liver-intestine or multivisceral transplant) at the Children’s Hospital of Pittsburgh and met criteria for evaluation for this study. Criteria to be met included sufficient length of time out from transplant (at least 6 months) and enough EBV load measurements to meet the definition of CHL. Of these 166 patients, 35 children (21%) met the criteria for chronic high EBV load carriers. From these thirty-five, 17 (49%) were EBV seronegative pre-transplant, 17 were EBV seropositive, and 1 (2%) child had unknown EBV serology status (Table 1).

The median time to onset of CHL carrier status was 6.25 months after transplant (range 0.5 to 101 months). (Table 2) In the patients who had been EBV seronegative prior to transplant, the mean time to onset was 5 months as compared to 6.25 months in the seropositive group, which was not statistically different. Seven of the seventeen patients (41%) who were EBV seronegative prior to transplant developed the onset of the CHL state at the time of primary infection.

Twelve children (34%) had a history of EBV disease (n=7, 20%) or PTLD (n=5, 14%) prior to meeting definition of CHL carrier state. A total of thirteen patients (37%) developed EBV disease after meeting the definition of high load carrier state with a median of 10 months of high load state before disease development (range: 6–29 months). EBV-related diseases diagnosed in this patient population included EBV enteritis (n=7, 20%), PTLD (n=4, 11%), EBV enteritis followed by tonsillitis (n=1, 3%) and EBV-positive spindle cell tumor (n=1, 3%). Disease was seen in 7/17 of the seronegative (1 PTLD) and 6/17 of the seropositive patients (3 PTLD). A total of four children died as a result of EBV-related disease after onset of CHL state while five other patients died of non-EBV related disease.

Unfortunately, EBV serologies were available from only 16 of 35 donors. Among these children, no specific pattern of increased risk could be identified based on donor EBV serology. Of nine donor EBV positive/recipient negative children with CHL, only four developed EBV with none developing PTLD. Five donor EBV positive/recipient EBV positive children had with CHL and three developed disease including one with PTLD. Of two donor EBV negative/recipient EBV negative patients, two developed EBV disease but neither developed PTLD. Similarly, no specific pattern was apparent among the 18 children whose EBV status was available pre-transplant but from whom donor EBV status was not

available. Two of seven donor not done/recipient negative children with CHL developed EBV disease including one with PTLD. Four of 11 donor not done/recipient EBV positive children developed EBV disease including two episodes of PTLD. The remaining child with CHL for whom neither donor nor recipient serologies were available pre-transplant did not develop disease.

Of interest, 63% of children did not develop EBV disease or PTLD during their high load carrier state. Further, thirteen patients (37%) resolved their CHL state without any apparent sequelae and of these children, two patients (6%) have had more than one episode of CHL without development of EBV-related disease. At this time, nine patients (26%) remain in CHL state without the development of any sequelae.

Discussion

With the development of technology leading to EBV load monitoring becoming standard practice, clinicians have been able to make informed decisions on immunosuppression regimen changes to prevent the development of PTLD. While elevated EBV loads are sensitive for the detection of patients at risk for progression to the development of EBV disease and PTLD, experience has shown that an elevated load is not specific for this outcome. As experience with EBV load monitoring has grown, a cohort of transplant recipients with chronically elevated levels of EBV has been identified. This finding has led to concerns and questions on the clinical significance of the elevated levels. We have previously reviewed and published our experience with chronic high EBV load carriers in our pediatric heart and liver transplant recipients at the Children's Hospital of Pittsburgh. Results of these studies identified very different outcomes of the EBV CHL state in these populations. A rate of 40% progression to PTLD was observed in our pediatric heart transplant recipients compared to 2.7% in our pediatric liver transplant recipients meeting the definition of the CHL state (8,15). The purpose of the current study was to review the experience and outcome of the CHL state in our pediatric intestinal transplant recipients.

Results of this study suggest that the outcome of the EBV CHL states for pediatric intestinal transplant recipients falls somewhere between outcomes observed in pediatric heart and liver transplant populations. We observed a rate of progression to PTLD of 11% in our pediatric intestinal transplant recipients. The differences in progression to PTLD in these three populations could be secondary to the level of immunosuppression that these patients received. Liver transplant patients, who have the lowest rate of progression to PTLD (2.7%), require the least amount of immunosuppressive drug therapy whereas heart transplant recipients, who need the most immunosuppression and also have the highest rate of progression amongst the three groups (45%) to PTLD (8,15). Small bowel transplant recipients require an intermediate level of immunosuppression compared to liver and heart transplant patients which correlates with their intermediate level of progression to PTLD. Thus, it is important to consider the level of immunosuppression that transplant patients are maintained on, especially in consideration of the patients' risk of developing PTLD.

Besides the frequency of progression to PTLD, intestinal transplant recipients differed from heart and liver transplant recipients in several additional ways. For the small bowel transplant population, half of the carriers were EBV seropositive prior to transplant. This is in contrast to our experience in pediatric liver or heart transplant recipients where it is the patients who develop primary infection post-transplant that are at the highest risk of becoming high load carriers. Another difference is the fact that for liver and heart transplant recipients, only those children who were EBV seronegative prior to transplantation typically progress to PTLD (8,15). In contrast, for pediatric intestinal transplant recipients, development of PTLD occurs in patients regardless of their pre-transplant EBV serostatus.

This difference, which has been previously reported about development of PTLD in general, also appeared to be true regarding progression to PTLD as a consequence of the EBV CHL state.

Several aspects of management unique to intestinal transplantation may affect our observations. Compared to liver and heart transplant patients, the small bowel is much easier to biopsy and thus, identify/diagnose EBV disease (i.e. enteritis). In many cases, the patients may not have been clinically symptomatic, however, surveillance biopsies led to their diagnosis. This may impact/bias the identification of EBV disease as it correlates to small bowel transplants in comparison to other solid organ transplant diseases. Further, in some of these patients, therapeutic intervention was provided, such as treatment with ganciclovir, IVIG or CMV IVIG. The beneficial effects of such treatments could have biased the results in such a way that fewer patients went on to develop PTLD or severe EBV disease. However, similar interventions were used in some cardiac and liver transplant patients with CHL status. It is unclear if these treatments impacts on progression of CHL to EBV disease. Accordingly, it is uncertain as to whether the increased frequency of use of these interventions affects the comparison of these comparison transplant populations with respect to CHL status and EBV disease.

This study provides insight into chronic high load EBV viral load carriage in a pediatric small intestine transplant population. However, as discussed within the text, there are other factors which may have affected the outcome of the patients and thus affect the interpretation of these results. As this study is a retrospective study, the results are subject to the limitations of such a study, such as a small sample size and clinical treatments employed in response to EBV viral load, such as changes in immunosuppression, use of IVIG and/or ganciclovir. In spite of the limitations of this study, we feel that these data along with data from previous studies can be used to help clinicians provide more informed decisions in the management of their transplant patients.

Acknowledgments

This work was presented in part at the 2008 American Transplant Congress in Toronto, ON, Canada, June 2008.
This work was supported in part by NIH Grant 5P50 HL074732 from the National Heart Lung and Blood Institute.

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Table 1

Comparison of chronic high load carriers in pediatric intestine, liver and heart transplant recipients

Organ Type (Years of patient accrual and follow-up)	Liver* (1997–2007)	Small Intestine (1994–2007)	Heart† (1989–2004)
No. of High Load Carriers	36	35	20
% Carriers Sero-negative Pre-Transplant	77%	49%	100%
% Hx Prior EBV Disease/PTLD	40%	34%	75%
Median Time of Onset of HLC State in months (Range)	4 (1–47)	6.25 (0.5–101)	Not Available
No. of Carriers progressing to PTLD	1 (2.7%)	4 (11%)#	9 (45%)

*Data abstracted from Green et al, Pediatric Transplantation (11) and

†Bingler et al., American Journal of Transplantation (9)

#One additional case of post-transplant spindle cell disease

Table 2

Epstein Barr virus (EBV) chronic high load carrier state by EBV serostatus prior to intestinal transplant

	Total	EBV negative Pre-Transplant	EBV positive Pre-Transplant
# of high load carriers (HLC)	35*	17	17
Median Time of Onset of HLC State in months (Range)	6.25 (0.5–101)	5 (0.5–52)	6.25 (0.75–90)
# EBV Disease Prior to HLC State (# PTLD)	12 (6)	4 (2)	7 (3)
# EBV Disease During HLC State (# PTLD)	13 (4)	7 (1)	6 (3)

* 1 Pt with unknown Pre-Tx EBV status who had PTLD prior to onset of HLC state