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Adenotonsillar Hypertrophy and Epstein-Barr Virus in Pediatric Organ Transplant Recipients

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Objectives/Hypothesis: **Epstein-Barr virus–related (EBV-related) lymphoid hyperplasia of the tonsils and adenoids is a precursor to post-transplantation lymphoproliferative disorder (PTLD). The incidence of post-transplantation adenotonsillar hypertrophy, a potential early sign of PTLD or EBV-related lymphoid hyperplasia, is not known. We sought to identify potential risk factors for adenotonsillar hypertrophy manifested as EBV-related hyperplasia and early PTLD in the pediatric solid organ transplant population.** ***Study Design:*** **Cross-sectional analysis.** ***Methods:*** **We developed a 65-point questionnaire concerning obstructive sleep disorder and upper respiratory tract infections and an 8-point focused physical examination, to identify prevalence of and risk factors for adenotonsillar hypertrophy in the pediatric transplant population. We evaluated 120 pediatric solid organ transplant recipients by parental questionnaire and focused adenotonsillar physical examination.** ***Results:*** **Of the 120 patients, 62 had undergone liver transplantation and 58 had undergone kidney transplantation. Overall, the mean questionnaire score was 8.36 (range, 0–40) and the mean physical examination score was 3.86 (range, 1–8). Patients whose EBV serological test result was negative at the time of transplant had higher scores for both the questionnaire (mean score, 10.24) and the physical examination (mean score, 4.56) than those whose EBV serological test result was positive at the time of transplantation (scores of 7.38 and 3.30 for questionnaire and physical examination, respectively). The difference in examination scores was statistically significant (*P *<.003).** ***Conclusions:*** **Epstein-Barr virus seronegativity at the time of organ transplantation is a known risk factor for PTLD, with associated risk of developing EBV-related lymphoid hyperplasia. Our results indicate a higher incidence of symptoms and signs consistent with adenotonsillar hypertrophy in the EBV seronegative population. Adenotonsillar hypertrophy may be a precursor to EBV-related lymphoid hyperplasia and PTLD and must be identified in this patient population.**
Key Words: **Adenotonsillar hypertrophy, Epstein-Barr virus, post-transplantation lymphoproliferative disorder, pediatric, transplant.**

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INTRODUCTION

The incidence of adenotonsillar hypertrophy (ATH) in the pediatric post-transplantation population is not known, and the etiology and clinical significance of such hypertrophy in this patient population have not been examined. Adenotonsillar enlargement in children who have had solid organ transplantation may be an early sign of post-transplantation lymphoproliferative disorder. Post-transplantation lymphoproliferative disorder is defined as the presence of abnormal proliferation of lymphoid cells and is associated with Epstein-Barr virus (EBV) infection in up to 80% of cases in the setting of immunosuppression. Prolonged use of immunosuppressants leads to a defect in T-cell regulation and in turn enables uncontrolled proliferation of B lymphocytes or T lymphocytes in response to viral (EBV) infection.[1] This may lead to a wide spectrum of PTLD-related diseases ranging from polyclonal lymphoid hyperplasia to monoclonal malignant lymphoma.[1,2] Lymphoproliferative disorders in post-transplantation patients tend to be more aggressive, respond poorly to chemotherapeutic agents, and have a poorer outcome compared with that of immunocompetent patients.[1]

Although more than 90% of healthy adults have been exposed to EBV infection, young children often have not yet been infected. Primary EBV infection in a healthy child or adolescent causes lymphoid enlargement secondary to EBV infection of B cells, causing massive B-cell proliferation.[3] This may induce minor symptoms of a febrile illness with cervical lymphadenopathy or adenotonsillar enlargement or both, or more significant symptoms consistent with infectious mononucleosis (fevers, lymphadenopathy, hepatosplenomegaly, adenotonsillar enlargement, fatigue).[3] Children receiving organ transplantation and subsequent immunosuppression may not have been

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How often does your child snore?
Never (0) Rarely (1) 1–4 times/mo (2) More than once a wk (3) Most nights (4)
How loud is the snoring?
No snoring (0) Mild/quiet (1) Medium loud (2) Loud (3) Extremely loud (4)
Does your child awaken in the middle of the night?
Never (0) Rarely (1) 1–4 times/mo (2) More than once a wk (3) Most nights (4)
Does your child wet the bed?
Never (0) Rarely (1) 1–4 times/mo (2) More than once a wk (3) Most nights (4)
Do you ever see your child struggling to breathe during sleep?
Yes (4) No (0)
Do you ever see your child stop breathing during sleep?
Yes (4) No (0)
When your child is asleep, do you ever shake him or her to start breathing again?
Yes (4) No (0)
Do you ever see your child's lips or skin turn blue or purple during sleep?
Yes (4) No (0)
Do you ever watch your child while he or she is asleep at night, afraid of his or her breathing?
Yes (4) No (0)
How often does your child have a sore throat?
Never (0) Rarely (1) 1–4 times/mo (2) More than once a wk (3) Almost daily (4)
How often does your child have sinusitis?
Never (0) Rarely (1) 1–4 times/mo (2) More than once a wk (3) Almost daily (4)
Does your child complain of morning headaches?
Never (0) Rarely (1) 1–4 times/mo (2) More than once a wk (3) Almost daily (4)
Is your child a daytime mouth-breather?
Never (0) Rarely (1) Frequently (2) Constantly (3)
Does your child have a problem with daytime sleepiness?
None (0) Slight (1) Moderate (2) Considerable (3) Very great (4)
Does your child have a problem with daytime hyperactivity?
None (0) Slight (1) Moderate (2) Considerable (3) Very great (4)
Does your child ever choke when eating solid food?
Never (0) Rarely (1) Frequently (2) Constantly (3)
Does your child ever choke when drinking liquids?
Never (0) Rarely (1) Frequently (2) Constantly (3)
(Maximum score: 65)

Fig. 1. Parental questionnaire.

exposed to EBV before organ transplantation. Epstein-Barr virus infection in these children may result in B-cell and T-cell proliferation proceeding unchecked, leading to EBV-related lymphoid hyperplasia or PTLD.[1,4,5] Uncontrolled proliferation of B lymphocytes infected with EBV may result in enlargement of the lymphoid tissue of Waldeyer's ring, most notably, the tonsils and adenoids.[3]

Epstein-Barr virus–related lymphoid hyperplasia or PTLD may first present as adenotonsillar enlargement. We sought to identify early signs and symptoms of adenotonsillar disease in children following solid organ transplantation. To date, there has not been a large-population analysis of this patient group to identify risk factors for EBV-related lymphoid hyperplasia and PTLD. Early diagnosis may lead to earlier intervention and diminished morbidity and mortality secondary to PTLD.

MATERIALS AND METHODS

We performed a cross-sectional analysis of children enrolled in the Mattel–University of California Los Angeles (UCLA) Children's Hospital Liver Transplantation and Kidney Transplantation Programs. After receiving Medical Institutional Review Board (IRB) approval from the UCLA Office for Protection of Human Research Subjects (IRB 99-10-010-01), we invited all pediatric patients attending the outpatient liver and kidney transplantation clinics to participate. All participants were invited during their routine follow-up post-transplantation clinic visits. Inclusion criteria included all patients 21 years of age or younger who have received kidney or liver transplantation. Exclusion criteria included children who had had adenotonsillectomy. Participants received a 65-point parental questionnaire (Fig. 1) and an 8-point focused physical examination (Fig. 2). The questionnaire was based on the obstructive sleep apnea 18-item questionnaire (OSA-18), which has been found to be a valid and reliable measure of symptoms of obstructive sleep apnea secondary to ATH.[6] We modified the questionnaire to attain more focused questions for this patient population. Because our patients all had chronic illness, we deleted questions from the OSA-18 related to missed activities, school behavior, and overall health because measures of these parameters may be influenced by their primary medical problem. The same examiner (A.M.S.), under the

Quiet respiration:

Mouth closed (0) Mouth open (1)

Intraoral examination:

Tonsils:

1+ (endophytic tonsils, anterior and posterior pillars visible) (1)

2+ (tonsils extend to tonsillar pillars) (2)

3+ (tonsils extend beyond tonsillar pillars, approximating uvula) (3)

4+ (tonsils approximating each other in the midline) (4)

Tonsillar asymmetry (4)

Intranasal examination:

Normal inferior turbinates/no discharge (0)

Edematous inferior turbinates (2)

Clear rhinorrhea (2)

Mucopurulent discharge (3)

(Maximum score: 8)

Fig. 2. Physical examination.

guidance of the senior author, performed all questionnaires and physical examinations. Objective data (Fig. 3) were obtained from the patient's hospital record.

Questionnaire and physical examination data were analyzed based on subjective and objective measures of signs and symptoms of ATH. Hospital medical record data were analyzed separately. Then, correlations between questionnaire, physical examination, and medical record data were analyzed.

RESULTS

We enrolled 120 patients (57 male and 63 female patients) over a 4-week period (June 5–June 29, 2000). Sixty-two patients had undergone liver transplantation, and 58 patients had undergone kidney transplantation. Of the liver transplant recipients, 57 patients received one transplant, 4 patients received two transplants, and 1 patient received three transplants before the time of this study. Fifty-three of the liver transplants were cadaveric, seven were from living-related donors, and eight had unavailable donor data. Of the kidney transplant recipients, 53 patients received one transplant, 4 patients received two transplants, and one patient received three transplants. Thirty-five of the kidney transplants were cadaveric, and 29 were from living-related donors. Mean age at the time of the study was 11.25 years (range, 0.75–21 y). Mean age at the time of transplantation was 7.33 years (range, 1 mo–20.4 y), and the mean number of years between transplantation and this study was 4.08 years (range, 0.75 mo–18.75 y). Immunosuppressant medications at the time of this study included cyclosporine, tacrolimus, prednisone, azathioprine, and mycophenolate. All patients were receiving between one and three immunosuppressant medications at the time of this study. In the group of kidney recipients, 42 patients were receiving cyclosporine, 16 patients were receiving tacrolimus, 58 patients were receiving prednisone, 4 patients were receiving azathioprine, and 44 patients were receiving mycophenolate. In the group of liver recipients, 4 patients were receiving cyclosporine, 57 patients were receiving tacrolimus, 38 patients were receiving prednisone, 5 patients were receiving azathioprine, and 15 patients were receiving mycophenolate (Table I).

The mean questionnaire score for all patients was 8.36 (SD ± 7.94; range, 0–40), and the mean physical examination score was 3.86 (SD ± 1.90; range, 1–8). In comparing liver transplant recipients and kidney transplant recipients, we found no significant difference between mean questionnaire scores (mean score for liver recipients was 8.68 [SD ± 7.84] with a range of 0–40; mean score for kidney recipients was 8.03 [SD ± 8.09] with a range of 0–38). In comparing mean physical examination scores of liver recipients and kidney recipients, there was also no significant difference (mean score for liver recipients was 4.02 [SD ± 1.71] with a range of 1–7; mean score for kidney recipients was 3.68 [SD ± 2.09] with a range of 1–8). Although both mean questionnaire score and mean physical examination score were slightly higher in the liver transplantation group than in the kidney transplantation group, these differences were not statistically significant.

Epstein-Barr virus serological status before transplantation was evaluated with respect to questionnaire and physical examination scores. Epstein-Barr virus serological values were based on the following parameters for antibody titers at our institution: EBV–viral capsid antigen (VCA) immunoglobulin M <1:20 = negative; EBV-VCA immunoglobulin M >1:20 = positive; EBV-VCA immunoglobulin G <1:10 = negative; EBV-VCA immunoglobulin G >1:10 and <1:640 = equivocal; EBV-VCA immunoglobulin G >1:640 or fourfold increase in antibody titer = positive; Epstein Barr nuclear antigen (EBNA) <1:5 = negative; EBNA >1:5 = positive. Data on pretransplantation EBV serological values was available for 118 of the 120 patients. Sixty-two patients (30 liver recipients and 32 kidney recipients) were EBV seropositive at the time of transplantation, 30 patients (17 liver recipients and 13 kidney recipients) were EBV seronegative at the time of transplantation, and 26 patients (17 liver recipients and 9 kidney recipients) had EBV serological results that were equivocal. The mean questionnaire

Name/medical record number:

Date of study:

Age:

Gender

Date of Birth:

Weight:

Height:

Date of transplantation:

Organ transplanted:

Donor: (living related/cadaver)

Reason for transplantation:

Donor EBV serology:

Recipient EBV serology:

Immunosuppressant medications:

History of organ rejection/multiple transplants (yes/no/number of transplants):

Fig. 3. Objective data. EBV = Epstein-Barr virus.

scores for these groups were 7.38 (SD ± 7.59; range, 0–40), 10.24 (SD ± 9.03, range, 0–38), and 8.19 (SD ± 6.01; range, 0–25) for the EBV seropositive, seronegative, and equivocal populations, respectively. The mean physical examination scores were 3.30 (SD ± 1.90; range, 1–8), 4.56 (SD ± 1.58; range, 2–7), and 4.31 (SD ± 1.72; range, 2–7) for these same groups. The difference between the mean physical examination scores for the EBV seronegative (4.56) and EBV seropositive (3.30) populations was statistically significant ($P < .003$) (Table II).

DISCUSSION

The role of EBV in the development of PTLD is well known.[7–10] As many as 90% of cases of PTLD in the early post-transplantation period are triggered by primary EBV infection.[4] The use of potent immunosuppressants such as OKT3, cyclosporine, and tacrolimus has led to an increase in the incidence of PTLD.[4,7,11,12] Post-transplantation lymphoproliferative disorder may often present in the region of Waldeyer's ring as ATH.[1,13] Thus, monitoring of Waldeyer's ring lymphoid hypertrophy may allow for early detection of EBV-related hyperplasia, a precursor of PTLD.

Epstein-Barr virus is a ubiquitous double-stranded DNA herpesvirus that infects B lymphocytes and induces polyclonal activation and proliferation.[14–16] In the immunocompetent host, after antibody response to infection, EBV-specific memory cytotoxic T lymphocytes control the lymphoproliferation, resulting in a self-limited process.[16] In immunosuppressed patients, the T-lymphocyte response is limited and EBV-induced polyclonal B cell proliferation may proceed unchecked.[16,17] Proliferation of B cells results in lymphoid enlargement. In its earliest form (EBV-related lymphoid hyperplasia), histological study reveals preservation of follicular architecture with variable hyperplasia of both follicular and interfollicular areas.[13] In situ hybridization reveals strong nuclear staining for EBNA. Histological features of PTLD include diffuse effacement of lymphoid follicles with polymorphous or monomorphous lymphocytic proliferation.[13]

Epstein-Barr virus seronegativity at the time of transplantation is a known risk factor for development of PTLD.[1,3,4,7,18–20] Thus, the highest-risk patients have been identified as those who are EBV seronegative at the time of transplantation with EBV seropositive donors. These patients are more likely to develop a primary EBV infection from the donor organ in the setting of immunosuppression. Iatrogenic immunosuppression limits the T-cell response that controls the proliferation of EBV-infected B cells, potentially leading to a spectrum of PTLD-related diseases.[2] A study of 40 pediatric liver transplant recipients at our institution revealed a fall in the incidence of EBV-related PTLD if EBV seronegative patients received preemptive intravenous ganciclovir at the time of transplantation.[7] Various risk factors have been identified by other groups, including tacrolimus use,[12] OKT3 use, cyclosporine use, and liver as opposed to kidney transplant.[4]

Previous studies have identified adenotonsillar tissue as a potential site of EBV-related lymphoid hyperplasia or PTLD.[13,21] In a recent retrospective review of children having undergone liver transplantation over a 14-year period (1984–1998), 39% of those who underwent adenotonsillectomy for airway obstruction or asymptomatic tonsillar enlargement were noted to have EBV-related lymphoid hyperplasia in the adenotonsillar tissue. One of the 13 patients who underwent adenotonsillectomy was noted to have large cell lymphoma of the adenotonsillar tissue. The authors emphasized that, given the high percentage of EBV-related hyperplasia in the tonsillar specimens of these patients, even asymptomatic adenotonsillar enlargement must be addressed aggressively in this patient population.[22] Thus, we sought to evaluate a large population of pediatric transplant recipients, focusing on both signs and symptoms of ATH. We found that children whose EBV serological finding was negative at the time of organ transplantation had higher questionnaire and physical examination scores than those who were seropositive at the time of transplantation. The difference in physical examination scores was statistically significant ($P < .003$). We did not find a statistically significant difference in comparing both questionnaire scores and physical examination scores for kidney versus liver transplant recipients. There was also no significant difference between the number of kidney transplant recipients and liver transplant recipients who were EBV seropositive (32 kidney recipients and 30 liver recipients) or EBV seronegative (13 kidney recipients and 17 liver recipients) at the time of organ transplantation.

CONCLUSION

Recipient EBV seronegativity at the time of organ transplant is a known risk factor for PTLD. Early forms of PTLD such as EBV-related lymphoid hyperplasia, as well as fulminant monomorphic PTLD, may present in the tonsils and adenoids. In the pediatric solid organ transplant population, monitoring adenotonsillar enlargement in the setting of immunosuppression may identify patients at risk for PTLD or EBV-related lymphoid hyperplasia of the tonsils and adenoids. In a cross-sectional analysis, we found that children who were EBV seronegative at the time of transplantation had more signs and symptoms of ATH than the EBV seropositive cohorts. This risk factor is consistent with findings of prior studies. Long-term surveillance of this population will further elucidate preemptive management options for these patients.

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BIBLIOGRAPHY

1. Dror Y, Greenberg M, Taylor G, et al. Lymphoproliferative disorders after organ transplantation in children. *Transplantation* 1999;67:990–998.

2. Harris NL, Ferry JA, Swerdlow SH. Posttransplant lymphoproliferative disorders: summary of society for hematopathology workshop. *Semin Diagn Pathol* 1997;14:8–14.
3. Fairley JW, Hunt BJ, Glover GW, Radley-Smith RC, Yacoub MH. Unusual lymphoproliferative oropharyngeal lesions in heart and heart-lung transplant recipients. *J Laryngol Otol* 1990;104:720–724.
4. Ho M. Risk factors and pathogenesis of posttransplant lymphoproliferative disorders. *Transplant Proc* 1995;27: 38–40.
5. Morrison VA, Dunn DL, Manivel JC, Gajl-Peczalska KJ, Peterson BA. Clinical characteristics of post-transplant lymphoproliferative disorders. *Am J Med* 1994:97:14.
6. Franco RA, Rosenfeld RM, Rao M. Quality of life for children with obstructive sleep apnea. *Otolaryngol Head Neck Surg* 2000;123:9–16.
7. McDiarmid SV, Jordan S, Lee GS, et al. Prevention and preemptive therapy of posttransplant lymphoproliferative disease in pediatric liver recipients. *Transplantation* 1998; 66:1604–1611.
8. Ho M, Jaffe R, Miller G, et al. The frequency of Epstein-Barr virus infection and associated lymphoproliferative syndrome after transplantation and its manifestations in children. *Transplantation* 1988;45:719–727.
9. Cacciarelli TV, Green M, Jaffe R. Management of posttransplant lymphoproliferative disease in pediatric liver transplant recipients receiving primary tacrolimus (FK506) therapy. *Transplantation* 1998;66:1047–1052.
10. Katz BZ, Raab-Traub N, Miller G. Latent and replicating forms of Epstein-Barr virus DNA in lymphomas and lymphoproliferative diseases. *J Infect Dis* 1989;160:589–598.
11. Myer CM, Reilly JS. Airway obstruction in an immunosuppressed child. *Arch Otolaryngol Head Neck Surg* 1985;111: 409–411.
12. Sokal EM, Antunes H, Beguin A, et al. Early signs and risk factors for the increased incidence of Epstein-Barr virus–related posttransplant lymphoproliferative diseases in pediatric liver transplant patients treated with tacrolimus. *Transplantation* 1997;64:1438–1442.
13. Huang RY, Shapiro NL. Adenotonsillar enlargement in pediatric patients following solid organ transplantation. *Arch Otolaryngol Head Neck Surg* 2000;126:159–164.
14. Thorley-Lawson DA. Immunological responses to Epstein-Barr virus infection and the pathogenesis of EBV-induced diseases. *Biochem Biophys Acta* 1988;948:263–286.
15. Miller G. Epstein-Barr virus: biology, pathogenesis, and medical aspects. In: Field BN, Knipe DM, eds. *Virology,* ed 2. New York: Raven Press, 1990:1921–1958.
16. Basgoz N, Preiksaitis JK. Post-transplant lymphoproliferative disorder. *Infect Dis Clin North Am* 1995;9:901–923.
17. Starzl TE, Porter KA, Iwatsuki S, et al. Reversibility of lymphomas and lymphoproliferative lesions developing under cyclosporine-steroid therapy. *Lancet* 1984;1:583–587.
18. Green M, Cacciarelli TV, Mazariegos GV, et al. Serial measurement of Epstein-Barr viral load in peripheral blood in pediatric liver transplant recipients during treatment for posttransplant lymphoproliferative disease. *Transplantation* 1998;66:1641–1644.
19. Lattyak BV, Rosenthal P, Mudge C, et al. Posttransplant lymphoproliferative disorder presenting in the head and neck. *Laryngoscope* 1998;108:1195–1198.
20. Boubenider S, Hiesse C, Goupy C, Kriaa F, Marchand S, Charpentier B. Incidence and consequences of posttransplantation lymphoproliferative disorders. *J Nephrol* 1997;10:136–145.
21. Lones MA, Mishalani S, Shintaku IP, Weiss LM, Nichols WS, Said JW. Changes in tonsils and adenoids in children with posttransplant lymphoproliferative disorder: report of three cases with early involvement of Waldeyer's ring. *Hum Pathol* 1995;26:525–530.
22. Broughton S, McClay JE, Murray A, et al. The effectiveness of tonsillectomy in diagnosing lymphoproliferative disease in pediatric patients after liver transplantation. *Arch Otolaryngol Head Neck Surg* 2000;126:1444–1447.