Posttransplant lymphoma in renal and heart allograft recipients: a single center experience

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ABSTRACT

Posttransplant lymphoproliferative disorder (PTLD) is a serious complication of organ transplantation, with a reported incidence between 0.8% to 32%. Herein we retrospectively analyzed the patients who diagnosed as PTLD in Akdeniz University. Within the 782 (773 renal and 9 heart) transplant recipients six patients were diagnosed as PTLD (diffuse large B-cell lymphoma). Five of them had renal, one had cardiac transplantation. Three patients were diagnosed within the first year of transplantation. Five patients had abdominal disease one had central nervous system involvement. All patients had positive Epstein-Barr virus (EBV) and cytomegalovirus (CMV) IgG at the time of diagnose. EBV-DNA with polymerase chain reaction (PCR) was found to be negative in five patients. Only one patient was survived after the diagnosis of PTLD. In conclusion, even with treatment the mortality rate is high in patients with PTLD. In order to decrease the incidence of PTLD and related mortality, the risk factors should be evaluated with multicenter studies.

Key Words: PTLD, EBV, CMV, Renal transplantation, Heart transplantation.

ÖZET

Böbrek ve kalp allograft alıcılarında posttransplant lenfoma: Tek merkez deneyimi

Transplantasyon sonrası gelişen lenfoproliferatif hastalık (PTLD) organ nakillerinin önemli bir kompleksasyonu olup, %0.8 ile %32 oranında görülmektedir. Burada Akdeniz Üniversitesi’nde tespit edilen PTLD olgularını retrospektif olarak değerlendirmeye amaçladık. Transplantasyon yapılmış olan 782 (773 böbrek, 9 kalp) hastanın altındaki difüz büyük B-hücreli lenfoma gözlemmiştir. Olguların beşinin böbrek, biri kalp nakli yapılmış hastalar olup, üç hasarda ilk bir yıl içinde lenfoma geliştiği tespit edilmiştir. Olguların beşinde hastalık batından, birinde santral sinir sisteminden kaynaklanmıştır. Tüm hastalarda lenfoma tanısı alındılar sıradaki sıraya Epstein-Barr virüs (EBV)
INTRODUCTION

Posttransplant lymphoproliferative disorder (PTLD) is a well-known complication of solid organ transplantation and it is an important cause of morbidity and mortality in transplant recipients. Typically, PTLD represents an uncontrolled proliferation of lymphoid cells from benign polyclonal lymphoid hyperplasia to aggressive monoclonal lymphoma[1,2].

Many probable risk factors have been identified for PTLD up to date. The type of graft, kind and intensity of immunosuppressive drugs, age of recipient, infectious agents particularly viruses [Epstein-Barr virus (EBV), cytomegalovirus (CMV), hepatitis C virus (HCV)], are some of the most important reported risk factors of this disorder[3-8]. Depending on the patients age groups and the type of allograft the incidence of PTLD varies between 0.8% to 32%. The highest rate was found in children with small-intestine transplants[9]. Herein we retrospectively analyzed the patients who diagnosed as PTLD in Akdeniz University.

MATERIALS and METHODS

Between January 1993 and July 2004, 782 (773 renal and 9 heart) patients underwent transplantation at Akdeniz University. The patients with PTLD were identified and their medical records were reviewed. Gender, sex, clinical presentation, immunosuppressive therapy, tumor characteristics and clinical outcome were evaluated.

For immunosuppression after transplantation, cadaveric organ recipients (220 patients) received polyclonal antithymocyte globulin (ATG 2.5 mg/kg/day for 7-14 days) as an induction therapy. As a maintenance therapy, cadaveric and living-related recipients got prednisolone (PRD), azathioprine (AZT) (100 mg/first day), mycophenolate mophetil (MMF) (2 g/per day) and cyclosporine A (CsA) (8 mg/per day) or tacrolimus (FK 506) (0.15 mg/first day). CsA and tacrolimus dosages were modulated according to blood levels. All patients received acyclovir (3.2 g/day according to glomerular filtration rate) therapy for six months after transplantation.

Patients were ordinarily seen in the outpatient clinic for two years. At each visit, physical examination, biochemical tests and whole blood counts, blood levels of CsA or FK 506 were determined. In addition the incidence of acute rejection episodes were recorded in every case.

The HCV, CMV and EBV serology of cardiac and renal transplant recipients were reviewed and we were able to reach most of them but we couldn’t obtain viral serology of cadaveric donors except human immunodeficiency virus (HIV).

The pathologic tissue specimens of patients were reevaluated and additionally if there was enough tissue immunohistochemistry for latent membrane protein (LMP) and immunophenotyping for LCA, CD3 and CD20 were performed. DNA was isolated from paraffin embedded tissue and EBV-DNA was searched with polymerase chain reaction (PCR) (EBV-Copies, quantitative determination of EBV-DNA, ARGENE-BIOSOFT, Varilhes, France).
RESULTS

In this study we identified six patients (five male and one female) with PTLD. Their median age was 40, five year (range 25-61 years). The clinical characteristics of patients were given on Table 1. Five of them had renal (0.6%), one had cardiac transplantation (11%). Two patients were transplanted cadaveric, four patients living related donor organ. The median duration from transplantation to diagnosis of lymphoma was 52 months (range 2-101 months). PTLD was diagnosed in three patients within the first year of transplantation. All of the patients with PTLD had positive EBV and CMV serology (IgG) before transplantation and just at the time of lymphoma diagnosis. Three patients developed positive CMV antigenemia after the transplantation. The HCV antibody was found to be negative in all PTLD patients.

The anti-HCV, EBV IgM and G, CMV IgM and G of transplanted patients with or without PTLD and donors were given on Table 2. CMV IgG and IgM were negative in two recipients and their donor. CMV IgG and M were negative in thirteen recipients but they received organ from IgG positive donors. One recipient was both EBV IgG and M negative but his donor had positive IgM and G antibodies.

Within 782 organ recipients 196 received cadaveric organ.

We obtained only five patients' pathological specimens and reevaluated them. The diagnosis was confirmed with morphological examination and with immunophenotyping studies and they were all identified as diffuse large B-cell lymphoma (CD20 positive). In addition, we searched for EBV-DNA with PCR on the pathologic specimen but we found them negative. We were able to perform immunohistochemistry for EBV LMP for only three specimens and all of them were found to be negative. Characteristics of

Table 1. The characteristics of patients with PTLD

<table>
<thead>
<tr>
<th>No</th>
<th>Age</th>
<th>Gender</th>
<th>Allograft</th>
<th>Etiology of disease</th>
<th>Donor</th>
<th>Immunosuppressive drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>M</td>
<td>Kidney</td>
<td>Polycystic renal disease</td>
<td>LR</td>
<td>PRD + CsA + MMF-AZT</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>F</td>
<td>Kidney</td>
<td>U</td>
<td>LR</td>
<td>PRD + CsA + MMF-AZT</td>
</tr>
<tr>
<td>3</td>
<td>63</td>
<td>M</td>
<td>Heart</td>
<td>Cardiomyopathy</td>
<td>C</td>
<td>PRD + ATG + CsA + MMF</td>
</tr>
<tr>
<td>4</td>
<td>49</td>
<td>M</td>
<td>Kidney</td>
<td>U</td>
<td>C</td>
<td>ATG + PRD + MMF + FK506</td>
</tr>
<tr>
<td>5</td>
<td>46</td>
<td>M</td>
<td>Kidney</td>
<td>Chronic glomerulonephritis</td>
<td>LR</td>
<td>AZT + PRD + CsA</td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>M</td>
<td>Kidney</td>
<td>Familial Mediterranean Fever-Amiloidozis</td>
<td>LR</td>
<td>PRD + AZT</td>
</tr>
</tbody>
</table>


Table 2. The viral serology of recipients, donors and PTLD patients and their donors

<table>
<thead>
<tr>
<th></th>
<th>Anti-HCV</th>
<th>EBV IgM</th>
<th>EBV IgG</th>
<th>CMV IgM</th>
<th>CMV IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>41</td>
<td>540</td>
<td>9</td>
<td>351</td>
<td>321</td>
<td>398</td>
</tr>
<tr>
<td>Donor</td>
<td>0</td>
<td>395</td>
<td>10</td>
<td>293</td>
<td>257</td>
</tr>
<tr>
<td>PTLD</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Donor</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

patients according to therapy, involved tissue and complications were given on Table 3.

Five patients were died due to disease progression or chemotherapy complications within six months. Only one patient who diagnosed as PTLD a year ago, is still alive.

DISCUSSION

Between 1973 and 2004 within the 782 allograft recipients six patients developed PTLD in our hospital. The incidence of PTLD was found to be consistent with the other studies which was found higher rates in cardiac transplantation than renal transplantation [11% (1/9) and 0.6% (5/773)] [9-13]. The highest rates were reported in T cell depleted bone-marrow and small intestine recipients. Though the immunosuppressive agents that were used nearly the same in all kind of transplantations the different rates probably evolved from the biologic differences of grafts.

It was reported that the donor recipient mismatch of EBV serostatus and seroconversion or primary infection of EBV after transplantation increase the risk of PTLD but EBV negative PTLD was also reported especially within the late occurring PTLD. In our study group, one recipient was both EBV IgG and M negative but his donor had positive IgM and G antibodies but this patient did not progress to PTLD. We had three early, three late occurring PTLD but, we could not able to detect EBV-DNA in the involved tissue of five of them with PCR. We used a kit for PCR which detects a fragment located in BNRF1 of EBV genome. EBV latent gene and antigen expression are different in different malignancies. For example African Burkitt’s lymphoma is lack of LMP antigen [14]. We don’t know if BNRF1 fragment of EBV-DNA is found in PTLD but we know that LMP is found in most of them. EBV LMP was studied by immunohistochemistry only two early onset and one late onset PTLD and all of them were found to be negative. EBV encoded RNA (EBER) with in situ hybridization and EBV-DNA with PCR are both powerful detection methods of EBV in tissues. We couldn’t perform EBER because of technical reasons and we studied EBV-DNA from paraffin embedded tissue. Isolated DNA was controlled in all tissues with a part of human genome but if there was sparse infiltration we may have not obtained involved tissue with lymphoma and so we could have not shown EBV-DNA. It was also reported that EBV-DNA was not amplify well from DNA which extracted paraffin embedded tissues

Table 3. The characteristics of PTLD patients according to therapy, involved tissue and complications

<table>
<thead>
<tr>
<th>No</th>
<th>Duration between transplantation and lymphoma (months)</th>
<th>Site of lymphoma</th>
<th>Treatment</th>
<th>Survive</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92</td>
<td>Stomach, liver</td>
<td>CHOP</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>77</td>
<td>Abdominal mass</td>
<td>CHOP</td>
<td>Dead</td>
<td>Pneumonia + gastrointestinal bleeding</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>Retroperitoneal mass, pleural effusion</td>
<td>CHOP</td>
<td>Dead</td>
<td>Sepsis</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>Spleen</td>
<td>CHOP</td>
<td>Dead</td>
<td>Hemophagocytic syndrome</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>Small bowel</td>
<td>CHOP</td>
<td>Dead</td>
<td>Sepsis</td>
</tr>
<tr>
<td>6</td>
<td>94</td>
<td>Cerebrum</td>
<td>CHOP</td>
<td>Dead</td>
<td>Intracranial bleeding</td>
</tr>
</tbody>
</table>

CHOP; C: Cyclophosphamide, H: doxorubicin HCL, O: Vincristine, P: Prednisolone.
and they try to optimize the reaction\cite{15}. And lastly probably whole EBV genome does not integrated into cellular components and so we could not amplify it. It is not possible to comment that all of the patients were EBV negative but there were nothing to implicate its association in our patients.

CMV association with PTLD has also been reported and it was shown that CMV itself has an immunosuppressive effect. Together with immunosuppressive agents CMV increases the risk of PTLD\cite{4}. Three of our patients had positive CMV antigenemia. Probably CMV was the most important causative agent in here but with only positive antigenemia it is probably obstacle to implicate their association.

The preventive effect of gancyclovir and following with acyclovir has been speculated\cite{16-18}. Our patients with PTLD had taken acyclovir for six months after the transplantation and additionally three of six patients also used gancyclovir for positive CMV antigenemia. According to our study antiviral agents are not preventive in transplant patients.

The immunosuppressive drugs were also accused for early PTLD. In 1969, it was first described as a complication of azathioprine based therapy by Penn et al\cite{19}. Many modifications in posttransplant management have been made over the last years. These include OKT3, ATG, MMF and FK506\cite{20}. Although the number of rejection episodes with the new agents was decreased, the incidence of PTLD did not change. All patients in this study had received STR + AZT or MMF + CsA or FK506 as a maintenance therapy. Additionally two patients received ATG also. Recently, Birkeland et al could not show the increased risk in any drug regime in the development of PTLD\cite{21}. Probably the immunosuppressive agents did not increase the risk in our patient group. As a result, because of our study population was too small it was not possible to find out any risk factor. But there may be different factors that increase the risk of PTLD. We can speculate that the regional factors such as environmental factors or polymorphisms of some genes like cytokines, MTHFR, may be different and they may increase the risk of PTLD.

PTLD mostly presents as a systemic disease involving lymphoid tissue, spleen, central nervous system or another extralymphoid region or sometimes allograft\cite{21,23}. None of the patients had palpable lymph node in present study. Three of our patients had gastrointestinal system involvement. Interestingly, we determined spontaneous splenic rupture due to involvement of lymphoma without enlargement of spleen in one of the patients who presented as early PTLD. This patient died with hemophagositic syndrome and disseminated intravascular coagulation. Two patients presented with abdominal discomfort due to a retroperitoneal mass. The other patient had CNS involvement. Although primary CNS lymphoma is relatively rare in general population, it is common in organ recipients and immunosuppressed patients\cite{24-26}. We should suspect CNS lymphoma whenever a transplant patient has neurological symptoms.

Despite numerous advances in this disease, the optimum treatment approach is not completely determined\cite{26,27}. Reduction of immunosuppressive therapy, antiviral drugs, chemotherapy and irradiation are the most important attempts. However, chemotherapy has considerable toxicities especially myelosuppression. Swinnen et al reported sepsis and cardiac toxicity depending chemotherapy drugs in their cardiac recipient\cite{28}. In this report, three of six PTLD patients died with infection (sepsis and pneumonia) as a complication of chemotherapy.

In conclusion, PTLD is one of the most important complications of organ transplantation and even with treatment, the mortality rate is high. In order to decrease PTLD and mortality the risk factors should be evaluated with multicenter studies.
REFERENCES


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