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SEVERE CYTOMEGALOVIRUS INFECTION AND IMMUNOSUPPRESSIVE THERAPY IN PEDIATRIC LIVER TRANSPLANTATION*

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Summary: Pehlivanoğlu E, Ament M.E, Spolidoro J.V.N, Vargas J, Busuttil R, Berquist W.E. (Departments of Pedatrics, Marmara University Faculty of Medicine, Istanbul, Turkey, and University of California, Los Angeles, USA). Severe cytomegalovirus infection and immunosuppressive therapy in pediatric liver transplantation. Turk J Pediatr 32: 3-11, 1990.

Twenty-two post-orthotropic liver transplant (OLT) recipients were studied to investigate the clinical, laboratory and histopathological differences between rejection and CMV infection. The mean age at the time of transplantation was five years. Nine of 22 (41 %) patients developed positive CMV, CF – IgG and IgM antibody titers and cultures for CMV following surgery, and three (group 1a) developed interstitial pneumonitis. CMV specific inclusion bodies were found in lung and liver biopsies. Two patients in group 1a were treated successfully with DHPG and decreasing immunosuppressive treatment, while the third died. Clinical presentation of rejection episodes were similar in all groups. CMV infected patients (group 1) received more transfusions of blood and blood products than the non-infected patients (group 2). Rejection episodes occurred sooner and more frequently in group 1a than in group 1b (CMV infected-asymptomatic) and group 2 (non-infected). Group 2 received fewer steroid boluses as well as azathioprine and OKT 3. A percutaneous liver biopsy with routine stains helped detect CMV when inclusion bodies were seen. We conclude that culture proven CMV infection is common post-OLT.

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Severe CMV infection occurred more frequently in those who had received greater doses of immunosuppressive therapy for possible graft rejection. Monitoring CMV infection following OLT is absolutely necessary. After OLT, decreasing the immunosuppressives and using antiviral agents are important in the management of CMV infection. ***Key words: cytomegalovirus infection, liver graft rejection, differential diagnosis, risk factors, treatment.***

Severe cytomegalovirus (CMV) infection is frequently detected in the immediate post - operative period following organ transplantation, at a time when the patient is subject to high - dose immunosuppressive therapy [1-8]. In liver transplant patients, the clinical manifestations of CMV infection may mimic liver graft rejection. Graft rejection, however, is treated with higher doses of immunosuppressive drugs. Since CMV viral replication and infection may be enhanced under these circumstances, the need for rapid and early detection of infection is necessary.

The aims of this study were (1) to determine the clinical and laboratory manifestations of CMV infection (2) to learn how CMV infection differs from liver graft rejection (3) to indicate the risk factors for developing CMV infection, and (4) to record the management and treatment of both conditions in post – orthotropic liver transplant (OLT) patients.

Material and Methods

Twenty-two patients who received OLT were retrospectively studied in the Department of Pediatrics, University of California, Los Angeles (UCLA) from March, 1984 to May, 1986. The mean age at the time of transplantation was five years and two months (range: seven months to 16 years). There were thirteen females and nine males. Pre-operative diagnoses of liver diseases were: biliary atresia (14 patients), alfa-1 antitrypsin deficiency (three patients), familial cholestasis syndrome (two patients), cryptogenic cirrhosis (one patient), hepatoblastoma (one patient) and leiomyoma with liver metastasis (one patient). All the patients were evaluated for CMV infection by serologic methods (complement fixation-IgG and CMV specific antibody) and/or urine, blood and throat cultures pre-operatively. If the patients developed clinical and/or biochemical evidence of graft rejection post-operatively, CMV serology was similarly obtained, as well as urine, throat, blood, and/or bronchia lavage for CMV cultures. Percutaneous liver biopsy was performed as indicated for diagnosis and management of presumed graft rejection. The biopsy, stained with hematoxylin and eosin, was also evaluated for CMV by routine light microscopy. The biopsies of the patients who had unexplained liver dysfunction, positive CMV cultures or interstitial pneumonitis were studied for CMV using the immunoperoxidase technique (Enzo Biochemical Inc, New York). All biopsy slides were reviewed by two pathologists to determine features of rejection and CMV infection.

Open lung biopsy and cultures were also performed in two cases who had positive cultures for CMV, poor response to anti-rejection therapy, and interstitial pneumonitis.

Rejection episodes were diagnosed clinically by the presence of fever, fatigue, jaundice, hepatomegaly, and right upper quadrant tenderness; biochemically by progressively increasing levels of SGOT (normal = 10-40 1 U/L), SGPT (normal = 15 1 U/L), total bilirubin (normal = 0-1.5 mg/dl), alkaline phosphatase (normal = 55-363 1 U/L), and histopathologically by liver biopsies revealing infiltration of portal tracts with plasma cells, lymphocytes, polymorphonuclear leukocytes, cholestasis, bile duct changes, cytoplasmic vacuolization, inflammatory infiltrate, centrilobular necrosis and vascular injury.

Initially all patients received methylprednisolone (20 mg/kg/day) as well as intravenous cyclosporine (2-3 mg/kg/day) before and after transplantation. The maintenance dosages for oral cyclosporine were 10-20 mg/kg/day, and for prednisone 1-2 mg/kg/day. The treatment regimen for rejection included either methylprednisolone pulses (15-20 mg/kg/day), Ortholone OKT 3 (monoclonal antibody for T cell; Ortho Pharmaceutical Corp, New Jersey) (2-5 mg/day i.v. bolus) or azathioprine (1-2 mg/kg/day). Routine immunosuppression and anti-rejection treatments were assessed for each patient. Treatment with 9-(1,3 dihydroxy-2 propoxymethyl) guanine (DHPG) (Syntex Research, Palo Alto, California) in a dose of 2.5 mg/kg q 8 hr for 10 days was given for CMV infection if the patient had CMV viremia and CMV related disease, such as interstitial pneumonia proven by lung biopsy.

The duration of hospitalization was determined for each patient following OLT.

Results

Forty-one percent of all patients (nine of 22; group 1) developed positive cultures (urine, blood, throat) 14 to 51 days (mean 22 days) following OLT. The number of patients in the infected and non-infected groups, and age and sex distributions are given in Table I. Three patients (13.5 %) in group 1 a (symptomatic group), also had a poor response to treatment during rejection episodes, and developed interstital pneumonitis 12 to 48 days after transplantation. One had an unexplained maculopapular skin rash. CMV specific inclusion bodies were found

TABLE I: Patient Profile

	Group 1 CMV infected	Group 2 Non-infected
Number	9	13
Age-mean (SD)	3.0 ±3.3	6 ±5.4
Sex-male/female	4/5	5/8

in the lung and liver biopsies of two cases. All the patients had fever (38-39 °C) with each rejection episode without positive cultures for infectious agents other than CMV. One patient developed gastrointestinal bleeding and became septic due to *E. coli and Pseudomonas*. She died four months after OLT, secondary to sepsis, gastric perforation and bleeding, and liver dysfunction. She had not received any anti-CMV treatment. The other two patients were treated with DHPG successfully for 10 days and steroid boluses were discontinued. Based on therapeutic blood levels, the oral cyclosporine dosage was 20-30 mg/kg/day and oral prednisone 1-2 mg/kg/day. In these patients clinical signs of pneumonia and skin rash improved markedly, and serum transaminase and bilirubin levels dropped to normal in 10 ten days, without additional immunosuppressive medication.

Six patients (group 1b; asymptomatic group) were found to have positive cultures from one or more sites. In four patients, the blood and saliva cultures were positive, whilst urine cultures were positive in all six patients. They did not develop CMV-related pneumonia, and neither was CMV detected in the liver tissue by culture or immunoperoxidase methods. All patients in group 1 had elevated complement-fixing antibody (CF) titers for CMV. Following DHPG treatment, two patients developed markedly higher titers than the pre-treatment levels.

Thirteen patients (group 2) did not have positive cultures for CMV. Laboratory data including culture and serology results, and mean values of liver function tests obtained during rejection episodes are given in Tables II and III. Group 1 patients received 59 ± 35 units of blood or blood products as compared with 25 ± 12 units in group 2 patients; these products were not tested for CMV.

TABLE II: Laboratory Data of Post-OLT Patients

	CMV infected		Non-infected
	Symptomatic	Asymptomatic	
	n:3	n:6	n:13
Cultures*	3	6	0
CF G* Pre-transplant	2	1	2
Post-transplant	3	6	3
IgM*	3	2	0

* No. of positive

TABLE III: Laboratory Data of Post-OLT Patients During Rejection Episodes*

	CMV infected		Non-infected
	Symptomatic	Asymptomatic	
	n:3	n:6	n:13
WBC (X 1000/mm^3)	11.3 ± 5.4	9.2 ± 3.9	14.4 ± 7.4
Serum albumin (g/dl)	3.0 ± 0.6	3.2 ± 0.3	3.5 ± 0.3
SGOT (1 U/L)	390 ±402	336 ±496	204 ±125
SGPT (1 U/L)	409 ±254	383 ±496	354 ±203
Total bilirubin (mg/dl)	4.4 ± 3.3	5.9 ± 5.2	4.2 ± 3.8
Conjugated bilirubin (mg/dl)	3.3 ± 2.9	4.8 ± 4.1	3.6 ± 3.1

* (mean SD)

Rejection episodes occurred in group 1a earlier, and more frequently, than in groups 1b and 2. CMV culture negative patients (group 2) received fewer steroid boluses as well as azathioprine and OKT 3. The data showing the number and onset of the rejection episodes, and the immunosuppressive therapy given for each patient in each group is shown in Table IV.

Clinical presentation of rejection episodes were similar in the three groups. All had fever, fatigue, right upper quadrant tenderness or hepatomegaly. In both groups 1b and 2 steroid boluses or OKT 3 relieved discomfort and fever, but in two patients in group 1, fever was elevated immediately following treatment.

Three types of rejection were found biochemically: 1) both transaminase and bilirubin levels elevated, 2) transaminase levels elevated predominantly, 3) bilirubin levels elevated predominantly. The patients with CMV in the liver mostly showed a Type I rejection pattern.

TABLE IV: Clinical Characteristics of Post-OLT Patients

	Group 1 CMV infected		Group 2 Non-infected
	Symptomatic	Asymptomatic	
	n:3	n:6	n:13
Onset of rejection episodes* (day)	5 ±1.0	7.5 ±2.0	8.5 ±5.0
No. of rejection episodes*	6.3 ±0.6	3.7 ±3.0	3.4 ±2.0
No. of steroid pulses*	5.7 ±0.5	3.5 ±3.0	3.3 ±2.0
No. of OKT 3 treated patients	3	2	4
No. of azathioprine treated patients	3	1	3

* mean (SD)

The liver biopsy findings given in Table V were not diagnostic for CMV in any of the groups unless CMV specific inclusion bodies were detected by immunoperoxidase. Intraparenchymal PMN clusters without surrounding inclusion bodies were found in one case from group 1, which is not typically seen in rejection. Hospitalization was longer in groups 1a and 1b than in group 2 (105 days; 57 days; 33 days, respectively).

TABLE V: Histologic Features of Liver Biopsies of Post-OLT Patients

	Group 1 CMV infected		Group 2 Non-infected
	Symptomatic	Asymptomatic	
	n:3	n:6	n:13
Portal infiltration	3	6	13
Cholestasis	3	6	6
Bile duct proliferation	–	1	2
Hepatocellular degeneration	3	2	1
Parenchymal mononuclear infiltration	1	2	1
Intraparenchymal polymorphonuclear leukocyte infiltration clusters	2	–	1
Inclusion bodies	2	–	–
Peroxidase staining	3	–	–

Discussion

The frequency of CMV infection following kidney transplantation has been reported to be up to 60 percent[9]. Comparable data for liver transplantation was not available until recently. Three centers, including our own, have recognized this complication, with one third of the patients infected. However, serious life-threatening infection is seen in 10-15 percent of patients[1-3].

The differentiation of liver rejection from CMV infection is very difficult because both conditions have similar symptoms and laboratory abnormalities. Since all OLT patients receive immunosuppressive medication to prevent rejection, they are more susceptible to acquire or reactivate CMV infection. Failure to correctly diagnose CMV infection may result in incorrect use of more immunosuppressive therapy which may result in complicated disseminated CMV infection and/or death.

Although there was no statistically significant difference in age between the infected and non-infected groups, the latter appeared older. This could be explained by the presence of protective antibodies for CMV from previous CMV infections in the older group. However, this condition may be a risk factor if the patient has been carrying the virus because of the possibility of reactivation of infection due to immunosuppression in the post-OLT period.

The level of immunosuppression was markedly higher in the patients who became infected with CMV. Although all transplant patients received cyclosporine, which acts on T cells and may promote CMV infection, group 1a also received a great number of methylprednisolone pulses and/or monoclonal antibody. The latter is recognized to promote CMV infection in transplant patients by increasing immunosuppression. In this study there was a correlation between the degree of immunosuppression and CMV infection. Another characteristic of those with CMV infection was the use of a fourth immunosuppressive agent, azathioprine. Although none of our patients had a positive culture for CMV prior to transplantation, two patients in group 1a had positive CMV specific CF titers, and were transfused with multiple units of blood and blood products, which are potential sources of CMV[10-12]. In our program, these products were not routinely tested for CMV.

In a comparable situation seen in renal transplantation, CMV infection is most often caused by a virus transferred by a transplanted kidney from a CMV antibody positive donor[13]. Knowing the donor's CMV status is important in order to predict the possibility of this complication.

Serologic tests can be useful in the diagnosis of CMV. Complement-fixing antibodies are typically elevated in infected children. However, they do not discriminate between passively acquired immunity and active infection[14]. In an immunocompromised patient, the antibody response to CMV infection will be different[15]. Only one-third of renal transplant patients produce CMV specific IgM antibodies with recurrent infections which can be useful in diagnosing active infection[16,17]. In our series, steadily increasing CF titers and positive IgM antibodies were found in all symptomatic patients with CMV disease; CF titers rose markedly following antiviral treatment with decreased immunosuppression. An accurate diagnosis of CMV in immunocompromised patients requires a combination of serologic markers and viral isolation[14,18].

During CMV infection, there are a wide range of histopathological changes which occur in the liver and are related to the immune status of the donor[8,15]. Although cytomegalic inclusion bodies were detected in two of our patents, who had clinical CMV disease, it was a late phenomenon[19,20]. The immune status of the patient, rapid degeneration of the hepatocytes, delayed diagnosis and sampling error in needle biopsies may be the factors explaining their absence[21]. CMV specific immunoperoxidase stain may be helpful in detecting CMV in tissues. Another specific diagnostic feature of a liver infected with CMV is granuloma-like clusters of polymorphonuclear leukocytes surrounding infected hepatocytes[19]. One of .our patients had such intraparenchymal PMN accumulation without inclusion body bearing cells. Although CMV has been reported as a cause of granulomatous hepatitis, none of our patients had this finding[15,22]. The

morphology of mild rejection episodes is no different from the changes observed in CMV infection, portal inflammation and cholestasis.

Clinical signs of rejection, fever, jaundice and fatigue, are indistinguishable from CMV infection. None of our infected patients developed retinitis but three of them had interstitial pneumonia which is common in serious CMV infection.

Laboratory manifestations of rejection episodes are similar to those of CMV hepatitis. Both conditions may cause elevated transaminase and bilirubin levels. In our CMV infected patients, who had CMV-related disease, the rejection reaction was different from the non-infected group. There was an abrupt elevation of transaminase and bilirubin levels with rejection that could be attributed to CMV. Several drugs and medical agents such as acyclovir, DHPG, hyperimmunoglobulin, and immunization have been used for treatment of CMV infection[23,24]. On the other hand, anti-rejection treatment affects the immune system and increases the possibility of exacerbation of the latent infection in those who carry the virus. When a patient has an active CMV infection, immunosuppressive treatment should be reduced, and administration of anti-viral drugs should be started to enable the patient to overcome the infection.

It is essential to know the status of the donor and monitor the recipient during pre-and post-transplant periods in order to decrease complications of immunosuppressive treatment. The ability to distinguish between CMV infection and rejection early in their course is an important factor in starting appropriate treatment and preventing unnecessary complications and death.

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