

Long-term follow-up of de novo allergy in pediatric liver transplantation – 10 yr experience of a single center

Lee Y, Lee YM, Kim MJ, Lee SK, Choe YH. Long-term follow-up of de novo allergy in pediatric liver transplantation – 10 yr experience of a single center.

Abstract: We conducted a study to clarify the incidence, clinical course, and risk factors of de novo allergies after liver transplantation. Ninety-three patients who had been followed longer than one yr and who had no previous allergy history were included. Forty-two patients (45.2%) developed de novo allergy. Of them, food allergy developed in 35 (37.6%). Respiratory allergy was observed in three (3.2%), and a patient (1.1%) had drug allergy. Fifty-two (55.9%) of the 93 patients developed eosinophilia. The median age of patients with de novo allergy was 15 months (IR 11.3–20 months). De novo allergy developed five months after liver transplantation (IR 2.3–9.5 months) and lasted for 16 months (IR 8–34.5 months). Younger age at liver transplantation displayed statistically significant differences in development of allergy between allergy and non-allergy groups. Twenty-nine (69.0%) patients improved from allergy during the follow-up period. No patient with de novo gastrointestinal allergy progressed to any respiratory allergy such as asthma. Older age at transplantation, EBV non-risk, and CMV non-risk had statistical significance in allergy improvement. Younger age at transplant predisposes to the development of allergy, while improvement of allergy is achieved more in older age.

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Tacrolimus is an 822-kDa macrolide antibiotic that has potent immunosuppressive properties. The mechanism of action is similar to that of cyclosporine in that it ultimately blocks the production of interleukin-2, thereby inhibiting further T-lymphocyte proliferation (1). It is widely used to prevent rejection after liver, kidney, and heart transplantation. However, a series of studies have shown that tacrolimus-based immune suppression is associated with serum IgE elevation, food allergies, and EGID (2–5). The primary immune suppression regimen of our institute's pediatric liver transplantation protocol

is tacrolimus-based immunosuppressive therapy. We have also experienced similar IgE elevation, development of EGID, and increased food allergies. Our first report on the development of eosinophilic colitis after pediatric liver transplantation from 2001 to 2005 was published in 2007 (6).

Pathogenesis of PLTA and role of tacrolimus remain unclear. Only some hypothetic explanations have been suggested and include tacrolimus influences on intestinal barrier function and intestinal permeability changes (7) or an imbalance of the Th1 and Th2 cytokine profile (5, 8). However, knowledge of risk factors, disease course, prognosis, and treatment modalities of PLTA is limited. Even our previous study had limitations concerning subject size and study period. Therefore, we presently conducted an observational study to clarify the incidence, clinical course, and risk factors of de novo allergies after liver transplantation.

Abbreviations: CAP, coated allergen particle; CMV, cytomegalovirus; EBV, Epstein–Barr virus; EGID, eosinophilic gastrointestinal disorder; HR, hazard ratio; IR, interquartile range; MAST, multiple antigen simultaneous test; PCR, polymerase chain reaction; PLTA, post-liver transplantation allergies; PTLT, post-transplantation lymphoproliferative disorder

Methods

Subjects

Our clinical experiences from 2001 to 2005 directed our attention to de novo allergies after liver transplantation. The prior publication dealt with mainly EGID (6). Since then, we have observed pediatric post-transplant patients, especially for allergic symptoms, according to the diagnosis and treatment protocol for allergic disease at post-transplantation follow-up. From May 2001 to May 2011, 130 cases of pediatric liver transplantation were performed at Samsung Medical Center, Seoul, Korea. Of 100 patients who had been followed longer than one yr, seven patients were excluded due to the previous allergy history. Therefore, 93 patients were included in our study protocol, and we collected various clinical data and laboratory findings.

History and clinical findings

Patient's previous family, allergy, and other medical history were collected before liver transplantation. The specific events including PTLT, rejection, graft failure, and allergic disorders were recorded. Patients and caregivers were questioned about symptoms when allergic disorder is suspected at hospital visit. Physical examinations were performed by pediatric gastrointestinal specialist or transplantation specialist. The follow-up intervals were variable according to the patient's condition.

Serum tacrolimus-level monitoring

Tacrolimus trough level was monitored according to our institute's transplantation protocol. Once tacrolimus level stabilized, monitoring intervals were lengthened. In our study, highest and lowest tacrolimus trough levels within two years after transplantation were noted and analyzed. The mean highest and lowest tacrolimus trough levels were calculated.

Virologic work-up

For EBV and CMV infection, the recipients who were virally naive and received viral positive liver were considered as high-risk group. After liver transplantation, viral markers were regularly followed by PCR.

Diagnosis and treatment of allergic disease

If patients had symptoms suspected of being due to allergic disease, such as urticaria, skin rash, and feeding intolerance, we checked total IgE level and presence of specific IgE by CAP test or MAST, in addition to their routine post-transplantation evaluation. Detailed medical history was obtained to specify potential allergens. Diagnosis of specific allergic disease was confirmed by pediatric gastrointestinal specialist or pediatric allergy specialist, on the basis of nature of the patients' symptoms, such as duration, treatment response, and relation with antigen exposure. For food allergy, once a potential allergen was specified, antigen restriction was tried. Symptom relief after antigen restriction was considered as strong evidence of food allergy. In some cases, food challenge tests were performed also. In case of infants without allergen specification, hypoallergenic formula was tried. Through the review of medical records, food allergy was defined on the basis of '2010 Guidelines for the Diagnosis and Management of Food Allergy in the United States' (9). We defined

post-liver transplantation eosinophilia positive, as an eosinophil count $>10\%$ and $>500/\text{mm}^3$ obtained more than once within follow-up period after liver transplantation (3).

The treatments were varied from just observation to strict antigen restriction or elemental diet, based on patients' age, clinical symptoms, and feeding methods. When a patient did not show allergic symptoms and/or signs any more under antigen rechallenge and normal activity without any kind of medical intervention (i.e., antigen restriction or medication); the patient was considered to have outgrown the de novo allergy.

Statistical analyses

For continuous data, a two-tailed Student's *t*-test, Mann-Whitney test, and Wilcoxon two-sample test were performed. For nominal data, the chi-square test and Fisher's exact test were performed. Values of $p < 0.05$ were considered significant. In addition, Cox proportional hazard analysis was performed to determine the HR of independent risk factors for allergy development and improvement. SAS version 9.1.3 (SAS Institute, Cary, NC, USA) was used for statistic analysis.

Results

General characteristics of subjects

The causative medical conditions of liver transplantation were biliary atresia ($n = 67$, 72.0%), fulminant liver failure ($n = 5$, 5.4%), Wilson disease ($n = 5$, 5.4%), Alagille syndrome ($n = 4$, 4.3%), and end-stage liver disease ($n = 3$, 3.2%). Nine subjects (9.7%) had miscellaneous conditions such as neoplastic or metabolic diseases. Thirty-six patients were male (38.7%). The median age of subjects at liver transplantation was 11 months (IR 8–34 months). Eight (8.6%) of 93 patients had allergic family history. The median follow-up duration was 70 months (IR, 41–90 months).

Virologic work-up

Before liver transplantation, 57 patients (61.3%) were at high risk of EBV infection, and 14 (15.1%) were at high risk of CMV infection. Seventy-three subjects (78.5%) had laboratory evidence of EBV infection after transplantation by PCR. Forty-four (47.3%) became CMV positive after transplantation.

Post-liver transplantation allergies

After liver transplantation, 42 patients (45.2%) had de novo allergy (Fig. 1). Of them, food allergy developed in 35 (83.3% of 42 and 37.6% of 93). The causative allergens confirmed by food challenge were found in 28 patients. Asthma, a respiratory allergy, was observed in three (7.1% and 3.2%), and a patient (2.4% and 1.1%) had drug allergy to antihepatitis B immunoglobulin.

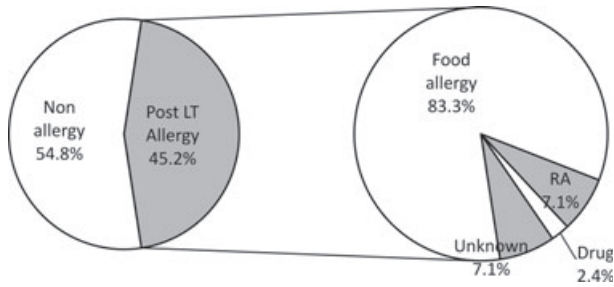


Fig. 1. Distribution of post-liver transplantation allergies based on causative allergens. LT, liver transplantation; RA, respiratory allergy.

No specific type of allergen was found in three (7.1%) with dermatologic manifestations of allergy. There was no patient with de novo food allergy that progressed to any respiratory allergy such as asthma. Five (11.9%) of 42 patients had allergic family history. The median age of patients with de novo allergy was 15 months (IR 11.3–20 months). In 22 (23.7% of 93) subjects, we found at least one specific IgE elevation. Milk was most common allergen (18 of 22 subjects), followed in order by egg white ($n = 14$), soy bean ($n = 11$), wheat ($n = 5$), and peanut ($n = 4$). Fifty-two (55.9%) of 93 patients developed eosinophilia. Among the clinical parameters and laboratory data collected, younger age at liver transplantation ($p = 0.001$), EBV high-risk group ($p = 0.007$), post-transplantation EBV infection ($p = 0.015$), and PTLN ($p = 0.005$) had statistically significant differences between the allergy and non-allergy groups. According to Cox proportional hazard analysis, younger age at liver transplantation remained significant for allergy development (Table 1).

Long-term follow-up of post-liver transplantation allergies

De novo allergy developed five months after liver transplantation (IR 2.3–9.5 months) and lasted for 16 months (IR 8–34.5 months). Twenty-nine (69.0%) showed improvement from allergy during follow-up period. There was a significant difference in age at liver transplantation ($p = 0.048$) and EBV high risk ($p = 0.038$) between allergy-improved and allergy-sustained group; improvement of allergy was achieved more in older age group and EBV non-risk group. The result of Cox proportional hazard analysis showed older age at transplantation, EBV non-risk, and CMV non-risk had statistical significance for improvement in allergy (Table 2).

Discussion

Many studies have reported the association of tacrolimus-based immunosuppression with food

Table 1. Cox proportional hazard analysis of risk factors in the development of post-liver transplantation allergy

Variables	HR (95.0% CI)	p
Age at LT	0.924 (.875–0.975)	0.004
Donor allergy	1.271 (.307–5.271)	0.741
Allergy Family hx	1.556 (.424–5.713)	0.505
EBV high risk	1.144 (.506–2.584)	0.747
CMV high risk	0.998 (.342–2.916)	0.998
Day when feeding started	0.941 (.706–1.254)	0.679
Highest tacrolimus trough level	0.999 (.940–1.062)	0.978
Lowest tacrolimus trough level	1.254 (.821–1.916)	0.294
Post-LT EBV	0.627 (.172–2.277)	0.478
Post-LT CMV	1.061 (.536–2.097)	0.865

Allergy Family hx, Family history of allergic disorder; EBV, Epstein–Barr virus; CMV, Cytomegalovirus; LT, liver transplantation; HR, Hazard Ratio; CI, Confidence Interval.

Table 2. Cox proportional hazard analysis of factors in the improvement of post-liver transplantation allergy

Variables	HR (95.0% CI)	p
Age at LT	1.231 (1.108–1.368)	0.000
Donor allergy	9.336 (.849–102.655)	0.068
Allergy family hx	0.234 (.028–1.951)	0.180
EBV high risk	0.168 (.040–0.701)	0.014
CMV high risk	0.074 (.007–0.773)	0.030
Day when feeding started	1.287 (.938–1.765)	0.118
Highest tacrolimus trough level	1.068 (.970–1.176)	0.180
Lowest tacrolimus trough level	0.478 (.141–1.621)	0.236
Post-LT EBV	3.927 (.578–26.685)	0.162
Post-LT CMV	0.885 (.262–2.987)	0.844
PTLD	0.791 (.191–3.279)	0.747
Number of sensitized antigen	0.734 (.370–1.453)	0.375

Improvement of allergy; symptom-free state without any kind of medical intervention and no more symptomatic aggravation after antigen rechallenge. Allergy family hx, family history of allergic disorder; EBV, Epstein–Barr virus; CMV, cytomegalovirus; PTLN, post-transplantation lymphoproliferative disorder; LT, liver transplantation; HR, hazard ratio; CI, confidence interval.

allergy in pediatric liver transplantation recipients, and the prevalence varies from 10% to 20% (2–5, 8, 10). In our study, de novo allergy was found in 45.2% and food allergy in 37.6% of the patients. The prevalence of allergic disorder in childhood is reported to range from 6.5% to 42.2%, on the basis of International Study of Asthma and Allergy in Childhood questionnaire studies (11–14). In the aspect of food allergy, the prevalence ranges from 3% to 35% in childhood (15). The reason why the prevalence rate of de novo allergy including food allergy in this study was relatively higher than those of normal population and other studies likely reflected our increased attention to allergy development in liver transplant patients since our 2001–2005 study (6). Other investigators might have neglected allergic symptoms because those symp-

toms are relatively common in children, and some spontaneously disappear in a short time. Second, some portion of de novo allergy in our study might have developed as a natural course, not being related with tacrolimus.

In post-liver transplantation eosinophilia, a similar tendency can be observed. It was 55.9% in our study and 28%–50% in other studies (3, 16). Granot et al. (3) attributed allergic symptoms in patients receiving tacrolimus to inhibition of interleukin (IL)-2 activation of T cells with resultant skewing of the immune response toward a Th2 response. The Th2 cytokines IL4 and IL5 promote production of IgE-secreting B cells and eosinophilia.

Boyle et al. (2) reported a case of liver transplant acquired food allergy in a child who received a split liver graft. The case was remarkable for the absence of new food allergy in the adult recipient of the same graft. They concluded that tacrolimus treatment alone cannot account for liver transplant acquired food allergy. Host factors such as the maturity of immune regulatory mechanisms are likely to play a critical role in the development of new food allergy after a liver transplant. Romero et al. (16) suggested that a younger age at transplantation, frequency of rejection episodes, tacrolimus-based immunosuppression, and EBV viral load might be associated with the development of this condition.

Recently, Shroff et al. (17) reported presentation of atopic disease in pediatric liver transplant recipients. The study showed lower incidence (14.2% vs. 45.2%) and later onset (19 months vs. five months after transplantation) of allergy in comparison with our study. The discrimination might be due to ethnic difference and/or younger median age at transplantation in our patients (16 months vs. 11 months).

Our study shows that the age at transplantation, EBV high risk, and mean highest tacrolimus trough level were factors associated with the development of de novo allergy. EBV and CMV may counteract the effects of each other on the immune system by their different Th1 and Th2 activations. CMV infection elicits a prototype Th1 response by inducing IL-2, tumor necrosis factor- α , and interferon- γ . EBV infection may transform human B cells, resulting in production of IL-5, which, in turn, may induce a chronic eosinophilic inflammation, promoting allergy and a preferred Th1 response (18–21). The mechanism by which EBV infection is directly associated with de novo allergy after transplantation still remains unclear. Moreover, EBV seroconversion mainly occurs

at the same period of allergy development after transplantation. Therefore, we should be cautious in interpretation of analysis for risk factors.

Another purpose of our study was to clarify the long-term clinical course and improving factors of de novo allergy after transplantation. In fact, persistently detectable sensitization to food over more than one yr in early childhood is a strong prognostic factor for subsequent allergic airway disease (22). This natural evolution of atopic disease is referred to as the “allergic march” (23). It is an important issue in clinical field, whether the allergy lasts for a long period and what makes the allergy last longer or shorter. According to Cox proportional hazard analysis in our study, older age at liver transplantation has a beneficial effect on allergy improvement. The current knowledge about food allergy gives information about a gradual age-related “growing out” from this type of allergy. The percentage of the children with the developed tolerance increased with age, from 2% after the first year of life, to 78.6% after the sixth year of life (24). In our study, two-thirds of the patients outgrew their allergies. This seems to be a relatively high rate. It is speculated that PLTA, at least some portion of them, might be a transient condition related to immunosuppression and/or EBV infection.

We found three interesting findings. First, more than two-thirds of patients outgrew their de novo allergy in a couple of years. Second, no newly developed gastrointestinal allergy progressed to allergic airway disease during the study period. Third, a recent Finnish study reported that the incidence of any positive food allergy test in children was threefold higher when both parents who had an allergic manifestation and twofold higher if either mother or father had allergic symptoms, compared with parental allergy-free group (25). However, the presence or absence of allergic family history in our subjects was not a risk factor for the development and improvement of de novo allergy. These results seem to be characteristic findings in de novo allergy after transplantation, compared with natural course of “allergic march” in general population.

It is well known that tacrolimus-based immunosuppression might play some role in development of PLTA. The limitation of the current study included the fact that we focused on only tacrolimus, even though immunosuppressants other than tacrolimus were also used for induction. Our study has another limitation that using

physician's diagnosis based on the patient's history and symptoms, not the gold standard for the diagnosis of these disorders, could introduce misclassification bias into our results and affect the internal validity of our study.

In conclusion, it is speculated that younger age at transplant predisposes to the development of allergy, while improvement of allergy is achieved more in older age and that other factors such as EBV high-risk group might play some roles in the pathogenesis. Further evaluation is necessary to confirm the clinical course of those cohorts with de novo allergy while they grow up to adulthood.

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Conflicts of interests

All authors report no conflicts.

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