Clinical characteristics of immune tolerance after pediatric liver transplantation

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Research article

Keywords: Immune tolerance, liver transplantation, biliary atresia, immunosuppression withdrawal

Posted Date: December 17th, 2019

DOI: https://doi.org/10.21203/rs.2.19066/v1

Abstract

Objective This study aimed to investigate the clinical characteristics of immune tolerance after pediatric liver transplantation and to identify the possible predictors.

Methods The clinical data from 37 cases of pediatric patients who underwent liver transplantation surgery in the Children's Hospital of Chongqing Medical University, China, were retrospectively analyzed. According to the status of the current immunosuppressant medications of the patients, they were divided into no-drug (n=4), single-drug (n=16) and multi-drug (n=17) groups. The possible influencing factors were screened based on these clinical data.

Results The factors that differed among the groups included the age at transplant and the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) of the transplant recipients. Between the single-drug group and the multi-drug group, the age, ALT level, and AST level of the transplant recipients were significantly different (P=0.01, P=0.00, and P=0.02, respectively); only the ALT level was significantly different (P=0.04) between the no-drug group and the multi-drug group. No significant difference was found in various factors between the no-drug and single-drug groups.

Conclusion The age of the recipient at transplant may be a potential factor affecting the generation of clinical immune tolerance in pediatric liver transplantation, while ALT and AST levels can be used as potential predictors of the generation of postoperative immune tolerance.

Background

Liver transplantation has been recognized as the most effective and ultimate treatment for irreversible acute or chronic liver disease, with satisfactory results in short- and long-term survival rates. However, similar to cases of the transplantation of many other organs, the overall life expectancy of liver transplant patients is still lower than that of the general population. This is primarily due to the compulsory lifelong use of immunosuppressant(s) to prevent transplant failure. The adverse reactions to these drugs include nephrotoxicity, diabetes, cardiovascular disease, metabolic syndrome, and bone loss. Furthermore, an opportunistic pathogen infection or cancer may potentially occur. The best way to solve these problems is to induce specific tolerance in the liver transplant recipient, thus achieving the long-term survival of the allograft without long-term immunosuppression.

Transplantation tolerance has several typical features. For example, the allograft can maintain normal function and morphology without using an immunosuppressant, thereby prolonging the allograft survival; an in vitro test reveals no or only a weak donor-specific reaction, and there is spontaneous acceptance of an allograft from a second-party donor with the rejection of an allograft from a third party [1]. Unlike acute or chronic rejection, our understanding of clinical immune tolerance is lacking. In some rare cases, such as when patients are non-compliant with immunosuppressive therapy, in cases of clinical trials in which the immunosuppressant is intentionally withdrawn, or in patients who have severe clinical considerations (lymphoproliferative disorder or life-threatening infections), the phenomenon of scattered tolerance can be observed. This phenomenon of clinical tolerance has gained increasing attention in the past 20 years. In addition, the results of a multi-center study indicated that immune tolerance was more common in children than in adults.

In clinical practice, we have also observed the tolerance of patients after withdrawing immunosuppressive drugs. To investigate the clinical characteristics affecting postoperative immune tolerance after pediatric liver transplantation and to identify possible predictors, the clinical data from 37 cases of pediatric patients who underwent liver transplantation surgery in the Children's Hospital of Chongqing Medical University, China, were retrospectively analyzed. The possible influencing factors were screened based on these clinical data, and a statistical analysis of these factors was performed.

Methods

Subjects

The subjects of this study were pediatric (< 18 years of age) recipients of a liver transplant with follow-up periods of at least two years after the transplantation. These pediatric patients underwent the liver transplantation surgery and follow-up management in the Children's Hospital of Chongqing Medical University and included 16 males and 21 females. The cases of death after the transplantation and the cases with missing follow-up data were excluded. All the patients were routinely followed up in our hospital, and the examination and medication data were recorded at each follow-up.

In this study, the four patients in the no-drug group withdrew the medication of their own accord and were not compliant with the prescription; these patients exhibited no obvious abnormalities in the indicators during the continuous follow-up period after stopping the medication. The current follow-up of all the pediatric patients showed normal graft function and no serious adverse events.

Methods

According to the current situation regarding immunosuppressant medications, the included cases were divided into no-drug (n = 4), single-drug (n = 16) and multi-drug (n = 17) groups. The patients in the single-drug group were receiving a single immunosuppressive drug therapy, the dosages of which were gradually reduced in accordance with a uniform standard based on the situation in the follow–up period, and most of the patients were taking close to the minimal dose. The patients in the multi-drug group were receiving two or more immunosuppressive drugs. The possible influencing factors were then screened based on the clinical data, which included the following: the age, gender, and blood type of the transplant recipient, the primary disease, the gender and age at transplantation of the donor, and the liver function indicators of the pediatric patients during the postoperative follow-up period, such as the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), λ-glutamyl transferase (GGT), total bilirubin (TBIL), direct bilirubin (DBIL), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH). These liver function indicators were obtained based on the average of the data from a previous follow-up. Finally, a statistical analysis of these factors was performed, and those factors with a significant difference in the comparisons among the groups were identified.

Statistical analysis

First, a univariate analysis was carried out for all the possible influencing factors, and the differences among the three groups were compared to initially identify the relevant factors. The measurement data used for this purpose were tested for normality according to the Kolmogorov-Smirnov method, and the normally distributed measurement data were then further tested for homogeneity of variance. The data exhibiting a normal distribution and homogeneity of variance were compared with an analysis of variance for multiple-sample means, while the rank sum test of multiple independent samples was performed for the non-normally distributed data. The countable data were compared using the χ^2 test of the R × C table, and Fisher's exact probability was calculated for those not satisfying the criteria of the χ^2 test. The categorical data were compared mainly using the rank sum test of multiple independent samples. The SPSS19.0 (IBM, Armonk, NY, USA) statistical software was used for the data processing. According to the significance level $\alpha = 0.05$, differences with $P < 0.05$ were considered statistically significant.

Results

General Information

The 37 cases of recipients included 16 males (43.2%) and 21 females (56.8%). The median age at liver transplantation was 7.6 months (interquartile range, 5.9–29.8 months). The 36 cases of donors included 12 males (32.4%) and 24 females (64.9%). There were nine cases of donor after cardiac death (DCD) (25.0%), including six males and three females, and one DCD donor donated liver to two recipients. There were 27 cases of related living donor liver transplantation (LDLT) (75.0%), including 20 mothers, six fathers, and one grandmother. The median age of the donors was 29.9 years (interquartile range, 22.5–35.9 years).

In this study, the primary diseases of the pediatric patients receiving liver transplantation included biliary atresia (n = 24, 64.9%), glycogen storage disease (n = 5, 13.5%), Wilson's disease (n = 2, 5.4%), and unexplained hepatic cirrhosis (n = 6, 16.2%) (Table 1).

Table 1

General information of the recipients and donors

Group	No.	Gender	Blood type	Primary disease	Age at transplant (months)	Transplant mode	Immunosuppressant(s) after transplant	Gender of donor	Age of donor (years)
No-drug	1	F	A	Biliary atresia	6.2	DCD	Prednisolone, Cyclosporine A	F	0.6
	2	F	A	Biliary atresia	8	LDLT	Prednisolone, Cyclosporine A	F	31.2
	3	F	B	Biliary atresia	7.5	LDLT	Prednisolone, Cyclosporine A	F	28.3
	4	M	B	Biliary atresia	7.2	LDLT	Prednisolone, Cyclosporine A	F	36
Single-drug	5	F	O	Biliary atresia	5.7	LDLT	Prednisolone, Cyclosporine A	M	24.3
	6	M	B	Biliary atresia	6.3	LDLT	Prednisolone, FK506, Cyclosporine A	F	36.8
	7	M	O	Biliary atresia	5	LDLT	Prednisolone, Cyclosporine A	F	31
	8	F	A	Biliary atresia	10.3	LDLT	Prednisolone, Cyclosporine A, Mycophenolate mofetil	M	31.8
	9	M	A	Biliary atresia	2.8	LDLT	Prednisolone, Cyclosporine A, Mycophenolate mofetil	F	26.7
	10	M	O	Biliary atresia	5.7	LDLT	Prednisolone, Cyclosporine A, Mycophenolate mofetil	F	42.3
	11		A	Biliary atresia	64.8	LDLT	Cyclosporine A, Mycophenolate mofetil	F	29.9
	12	M	O	Glycogen storage disease	5.7	LDLT	Prednisolone, FK506, Mycophenolate mofetil	F	42.8
	13	M	B	Unexplained hepatic cirrhosis	7.2	LDLT	Prednisolone, Cyclosporine A	F	2.2
	14	M	O	Unexplained hepatic cirrhosis	4.7	DCD	Prednisolone, FK506, Cyclosporine A	M	5
	15	F	AB	Biliary atresia	7.6	DCD	Prednisolone, FK506, Cyclosporine A	M	36
	16	F	B	Biliary atresia	6	LDLT	Prednisolone, Cyclosporine A	F	37.3
	17	F	A	Biliary atresia	9.1	LDLT	Prednisolone, Cyclosporine A	F	9.1
	18	F	B	Biliary atresia	6.1	DCD	Prednisolone, Cyclosporine A, Mycophenolate mofetil	F	22.8

Group	No.	Gender	Blood type	Primary disease	Age at transplant (months)	Transplant mode	Immunosuppressant(s) after transplant	Gender of donor	Age of donor (years)
	19	F	A	Biliary atresia	156.6	DCD	Prednisolone, Cyclosporine A, Mycophenolate mofetil	M	42
	20	M	A	Wilson's disease	5	LDLT	Prednisolone, FK506, Mycophenolate mofetil	F	28.5
Multi-drug	21	M	B	Unexplained hepatic cirrhosis	108.9	LDLT	Prednisolone, FK506, Mycophenolate mofetil	F	28
	22	F	AB	Glycogen storage disease	31.6	LDLT	Prednisolone, FK506	M	30.3
	23	M	A	Biliary atresia	65.4	LDLT	Prednisolone, FK506, Cyclosporine A	F	31.8
	24	M	O	Biliary atresia	9.4	LDLT	Prednisolone, Cyclosporine A	F	33.3
	25	M	A	Unexplained hepatic cirrhosis	5	LDLT	Prednisolone, Cyclosporine A	F	29.3
	26	F	O	Biliary atresia	6.4	DCD	Prednisolone, FK506, Cyclosporine A, Mycophenolate mofetil	M	3
	27	F	O	Wilson's disease	19.1	LDLT	Prednisolone, FK506, Mycophenolate mofetil	M	35.8
	28	M	B	Biliary atresia	151.2	LDLT	Prednisolone, Cyclosporine A	M	34
	29	F	AB	Glycogen storage disease	14.5	LDLT	Prednisolone, Cyclosporine A	F	32.3
	30	F	O	Glycogen storage disease	44	LDLT	Prednisolone, FK506, Mycophenolate mofetil	F	39.3
	31	F	O	Biliary atresia	28	LDLT	Prednisolone, Cyclosporine A	M	22.2
	32	F	AB	Biliary atresia	7.4	DCD	Prednisolone, FK506, Cyclosporine A	F	1.3
	33	F	B	Biliary atresia	27.5	LDLT	Prednisolone, Cyclosporine A, Mycophenolate mofetil	F	29.9
	34	M	AB	Biliary atresia	10.6	LDLT	Prednisolone, Cyclosporine A, Mycophenolate mofetil	F	36.4
	35	F	AB	Unexplained hepatic cirrhosis	4.8	DCD	Prednisolone, Cyclosporine A	M	0.4
	36	M	O	Unexplained hepatic cirrhosis	86.7	DCD	Prednisolone, FK506, Mycophenolate mofetil	M	4.8

Group	No.	Gender	Blood type	Primary disease	Age at transplant (months)	Transplant mode	Immunosuppressant(s) after transplant	Gender of donor	Age of donor (years)
	37	F	A	Glycogen storage disease	124	DCD	Prednisolone, FK506, Mycophenolate mofetil	M	22.8

Gender, blood type, transplant mode and primary disease

In this study, Fisher's exact test was performed for the four countable data including gender, blood type, transplant mode and primary disease. The analysis revealed no statistically significant differences in these factors (Table 2).

Table 2
Gender, blood type, transplant mode and primary disease

Factor	Level	No-drug (n = 4)	Single-drug (n = 16)	Multi-drug (n = 17)	Fisher's exact test value	P
Recipient's gender	M	1(25.0%)	5(31.3%)	8(47.1%)	1.17	0.62
	F	3(75.0%)	11(47.1%)	9(52.9%)		
Recipient's blood type	A	2(50.0%)	6(37.5%)	3(17.6%)	7.09	0.27
	B	2(50.0%)	4(25.0%)	3(17.6%)		
	O	0	5(31.3%)	6(35.3%)		
	AB	0	1(6.3%)	5(29.4%)		
Donor's gender	M	0	5(31.3%)	8(47.1%)	2.93	0.24
	F	4(100.0%)	11(68.7%)	9(52.9%)		
Transplant mode	LDLT	3(75.0%)	12(75.0%)	12(70.6%)	0.28	1.00
	DCD	1(25.0%)	4(25.0%)	5(29.4%)		
Primary disease	Biliary atresia	4(100.0%)	12(75.0%)	8(47.1%)	5.07	0.50
	Glycogen storage disease	0	1(6.3%)	4(23.5%)		
	Wilson's disease	0	1(6.3%)	1(5.9%)		
	Unexplained hepatic cirrhosis	0	2(12.5%)	4(23.5%)		

Age of the donor and recipient at transplantation

The Kruskal-Wallis test was conducted for the age of the donor and recipient at transplant, and the results indicated a statistically significant difference in the age of the recipients (P = 0.011). Further pairwise comparisons between the groups revealed a statistically significant difference in the age of the recipients at transplant between the single-drug group and the multi-drug group (P = 0.010) and no statistically significant difference between the no-drug and single-drug groups and between the no-drug and multi-drug groups (Table 3, Table 4).

Table 3
Age at transplant and liver function

	Median	Standard deviation	Percentile			chi-square	df	P
			25	50	75			
Age of the donor at transplant (years)	29.90	13.12	22.50	29.90	35.90	0.97	2	0.62
Age of the recipient at transplant (months)	7.60	42.55	5.85	7.60	29.80	8.96	2	0.01*
ALT (U/L)	43.25	49.36	31.39	43.25	78.43	14.16	2	0.00*
AST (U/L)	49.97	30.03	37.56	49.97	72.71	8.38	2	0.02*
GGT (U/L)	37.18	173.86	23.32	37.18	114.15	2.80	2	0.25
TBIL (μmol/L)	12.52	13.02	10.52	12.52	15.57	5.01	2	0.08
DBIL (μmol/L)	4.10	9.28	2.74	4.10	6.08	4.45	2	0.11
ALP (μmol/L)	282.64	193.82	227.99	282.64	405.59	0.87	2	0.65

Table 4
Pairwise comparisons of the age of the recipient at transplant and the levels of ALT and AST between groups

Factor	Comparison	Test statistic	Standard error	Standard test statistic	P
Age of the recipient at transplant	1 vs. 2	2.78	6.05	0.46	1.00
	1 vs. 3	-8.33	6.01	-1.39	0.49
	2 vs. 3	-11.11	3.77	-2.95	0.01*
ALT	1 vs. 2	-2.00	6.05	-0.33	1.00
	1 vs. 3	-14.99	6.02	-2.49	0.04*
	2 vs. 3	-12.99	3.77	-3.44	0.00*
AST	1 vs. 2	0.00	6.05	0.00	1.00
	1 vs. 3	-10.34	6.02	-1.72	0.26
	2 vs. 3	-10.34	3.77	-2.74	0.02*

Indicators of liver function

The indicators of liver function, including the levels of ALT, AST, GGT, TBIL, DBIL, and ALP, did not meet the requirement of normal distribution. The Kruskal-Wallis test was performed, and the results showed statistically significant differences in the ALT and AST levels (P = 0.00 and P = 0.02, respectively). The further pairwise comparisons between groups found statistically significant differences in the levels of ALT between the no-drug group and the multi-drug group (P = 0.04) and between the single-drug group and the multi-drug group (P = 0.00). The AST level exhibited a statistically significant difference between the single-drug and multi-drug groups (P = 0.02). The level of LDH was normally distributed (P = 0.20), and the results of a further Lavene test for the homogeneity of variance were positive (P = 0.22). An analysis of variance using the SNK-q test method found no statistically significant difference in the LDH levels among the three groups (P = 0.57) (Table 3, Table 4).

Discussion

Immune tolerance is considered the "Holy Grail" in transplant medicine. Although there are many successful methods to induce tolerance in rodents, these approaches are rarely effective in primates or humans. "Operational tolerance" or "functional tolerance" refers to a case in which no specific destructive immune response against the graft is detected without the use of any immunosuppressive drug. "Prope tolerance" refers to the maintenance of the stable function of a graft by using a minimal dose of an immunosuppressive agent [2]. In addition, there are many other definitions of tolerance. Nevertheless, no consensus has been reached on what is considered stable function or how long the maintenance of stable function must be for a definition of tolerance.

In this study, the four pediatric patients in the no-drug group withdrew the medication of their own accord without following the doctor's advice. The continuous follow-up after the withdrawal revealed no abnormality in various indicators, suggesting that the graft function was normal with no rejection or serious adverse events. Accordingly, we believe that these four patients achieved the clinical criteria of operational tolerance and that a state of tolerance had been established. The 16 patients in the single-drug group were taking only a single immunosuppressant at a dosage that was mostly close to the minimum dose, while maintaining normal graft function; therefore, these patients can be considered to have generated prope tolerance. Thus, both the no-drug group and the single-drug group can generally be considered to be in a state of immune tolerance, and only the extent of tolerance was different.

A research team in Pittsburgh described immune tolerance for the first time in 1990 [3, 4]. Later, the Hospital of Tokyo University reported that 88 out of 581 cases of pediatric related LDLT recipients (15%) displayed immune tolerance [5]. In 2012, the University of California conducted a prospective, multicenter, and open-label pilot study of 20 cases of pediatric LDLT recipients with stable disease, and 60% of the pediatric LDLT recipients maintained normal function and stable morphology of the graft after the withdrawal of immunosuppression for at least one year, thus achieving immune tolerance [6]. In addition, the postoperative termination of immunosuppression after a transplant has also randomly been reported in many countries.

In this study, a statistical analysis was performed of the clinical data from 37 cases of pediatric patients who underwent a liver transplantation, and the results revealed differences in three factors, including the age of the recipients at transplant and the ALT and AST levels, among the different groups. Between the single-drug group and the multi-drug group, the age of the recipients at transplant and the levels of ALT and AST were all significantly different, but only the ALT level was significantly different between the no-drug group and the multi-drug group, while the no-drug and single-drug groups showed no significant difference in any of the three factors. Accordingly, we can conclude that the age of the recipient at transplantation may be a potential factor influencing the generation of clinical immune tolerance in pediatric liver transplantation, while the ALT and AST levels can be used as potential predictors of the generation of clinical immune tolerance.

Although the number of patients included in this study was small, especially the number of patients in the no-drug group, a few factors that may be associated with immune tolerance were still found. The age of the recipients at transplant was different between the single-drug group and the multi-drug group, suggesting that it may be a potential factor influencing the generation of tolerance in pediatric patients. This may be because the immune system gradually matures with increasing age. No evidence for a difference in age was found between the no-drug group and the multi-drug group, which may be due to the small sample size of the no-drug group. As important indicators of liver function, ALT and AST are affected by many factors, such as the function of the liver itself, drugs, and complications. These enzymes may be used as potential predictors of immune tolerance, which should be given more attention in the future in the follow-up of pediatric patients.

In addition, we found that the primary diseases of the pediatric patients in the no-drug group were all biliary atresia, and the donors were all female. Additionally, most cases of liver transplantation were maternal LDLT (3/4). The primary diseases of the pediatric patients in the single-drug group were also mostly biliary atresia, and the donors were mostly female, with the majority of cases being maternal LDLT. Although the analysis showed that these factors were not significantly different among the groups, this result may be because of the small sample size. In a future study, a multi-center large-scale data analysis should be performed to further explore whether these factors are related to the generation of immune tolerance.

Conclusions

The present data suggested that the generation of clinical immune tolerance in pediatric liver transplantation might be influenced by the age of the recipient at transplantation. ALT and AST levels can be treated as the potential predictors of clinical immune tolerance. In future studies, multicenter large-scale data analysis should be performed to further explore whether these factors are related to the development of immune tolerance.

Abbreviations

ALT:alanine aminotransferase AST:aspartate aminotransferase GGT:λ-glutamyl transferase, TBIL:total bilirubin DBIL:direct bilirubin ALP:alkaline phosphatase LDH:lactate dehydrogenase DCD:donor after cardiac death LDLT:living donor liver transplantation

Declarations

Ethics approval and consent to participate: This study was approved by the ethics committee of Chongqing Medical University, and was performed in accordance with the Helsinki Declaration of 1975, as revised in1983. All the patients enrolled were comprehensively informed, and written informed consent to participate in this research and publish the data were obtained. Consent was obtained from a parent or guardian on behalf of any participants under the age of 16.

Consent for publication: Not applicable

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests : No potential conflicts of interest relevant to this article are reported.

Funding **:** The research was supported by National Natural Science Foundation of China (No: 30973440, 30770950), and the key project of the Chongqing Natural Science Foundation (CSTC, 2008BA0021, cstc2012jjA0155).

Author Contributions: Bailin Chen designed and analyzed the data and evaluated the manuscript. Chunbao Guo analyzed the data and wrote the paper. All authors have read and approved the manuscript

Acknowledgements: We thank Prof. Xianqing Jin for providing technical assistance and for insightful discussions during the preparation of the manuscript. We thank Dr Xiaoyong Zhang at the Wistar Institute, USA, for help with the linguistic revision of the manuscript.

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