의학석사 학위논문

Clinical Significance of Graft Fibrosis and Fatty Change Based on Late Protocol Liver Biopsy after Liver Transplantation

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Clinical Significance of Graft Fibrosis and Fatty Change Based on Late Protocol Liver Biopsy after Liver Transplantation

(- Fibrosis and Fatty Change Liver Recipient Based on Late Protocol Biopsy -)

by

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ABSTRACT

Introduction: Little is known about the results of late protocol liver biopsy after LT yet, especially in aspect of graft fibrosis and fatty change. The aim of this study was to evaluate graft fibrosis and fatty change based on late protocol biopsy after LT, and to investigate possible relationships between clinical data and graft fibrosis or fatty change.

Patients and Methods: We retrospectively reviewed the recipients who underwent late protocol liver biopsy (> 1 year after LT) at our center between August 2010 and August 2012. Sono-guided fine needle aspiration liver biopsies and hematoxylin and eosin stains and Masson trichrome stains were performed. The METAVIR system for staging of graft fibrosis and the steatosis scoring system devised by the Pathology Committee of the NASH Clinical Research Network for fatty change grading were used. We analyzed the related peri-transplant clinical data and fibrosis or fatty change based on liver biopsy.

Results: Total 174 late protocol liver biopsies were done in 131 adult patients. Graft fibrosis was 23.6% (n = 41/174). Among them, significant (moderate and more) fibrosis was 46.3% (n = 19/41). In multivariate analysis between the group without fibrosis and the other group with fibrosis, based on the significant variables in univariate analysis, high mean fasting blood sugar (FBS, ≥ 126 mg/dL) during 6 months before biopsy regardless of treatment of DM (risk ratio 7.260, $p = 0.001$) and positive ductular reaction in CK-19 (risk

ratio 7.931, $p < 0.001$) were significant factors for graft fibrosis after 1 year from LT. For significant fibrosis, positive ductular reaction in CK-19 (risk ratio 20.335, $p < 0.001$) and positive bile duct damage in CK-19 (risk ratio 17.351, $p = 0.001$) were strongly significant factors.

Fatty change showed in 25.3% (n = 44/174). Among them, significant change was 31.8% (n = 14/44). With multivariate analysis based on the significant variables in univariate analysis, male sex (risk ratio 3.448, $p = 0.037$), alcohol history before LT (risk ratio 5.755, $p = 0.013$), BMI ≥ 25 kg/m^2 (risk ratio 2.926, $p = 0.020$) were clinically related factors for late graft fatty change. For significant fatty change, BMI ≥ 25 kg/m^2 at biopsy showed significant difference (risk ratio 3.481, $p = 0.037$).

Conclusions: In conclusion, the graft fibrosis and fatty change based on late protocol biopsy after LT comprised a considerable portion. In this study, high mean FBS (≥ 126 mg/dL) during 6 months before biopsy regardless of treatment of DM and positive ductular reaction in CK-19 were closely related to the graft fibrosis, and male, alcohol history before LT and obesity (BMI ≥ 25 kg/m^2) at biopsy were related to graft fatty change. We can apply these results to clinical management for recipients and that may be a good influence to future long-term outcome of the patients.

Key words: liver protocol biopsy, graft fibrosis, fatty change, liver transplantation

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Introduction

Hepatic fibrosis is the deposition of excess extracelluar matrix that is rich in fibril-forming collagens. In the vast majority of cases, hepatic fibrosis is the result of chronic liver diseases and assessment of fibrosis is very important including hepatitis C virus (HCV) infection. In the case of liver transplantation (LT), graft fibrosis is also important issue for graft function including specific case of recurred viral disease such as HCV or chronic rejection. Despite of limitations related to sampling and interpretation, histologic examination remains the gold standard for staging chronic liver disease and hepatic fibrosis for now (1).

Meanwhile, nonalcoholic fatty liver disease (NAFLD) affects a substantial proportion of the general population worldwide and there have been an increasing prevalence and interests recently, especially, in terms of association with obesity and metabolic syndrome. In case of LT, recipients are at risk for developing a number of features of the metabolic syndrome, such as diabetes mellitus (DM), weight gain, hypertension, and hyperlipidemia and are thus predisposed to the development of NAFLD and more common than in the general population (2-5). Fatty change is present in 18~40% of post-LT biopsies, and is also a common finding in protocol biopsies obtained from patiets with normal liver function tests in several studies (6-8).

As outcomes of LT have improved, the major concern in clinical management of the recipient has been shifting from prevention of early acute rejection to the maintenance of late and long-term graft survival and the

reduction of side-effects associated with immunosuppressive agents (9). However, biochemistry such as liver function test and non-invasive markers can not accurately reflect the graft function as much as liver biopsy, and have been known as being unreliable comparing to graft histology so far (10). In this respect, recently, there are several reports about the usefulness of late protocol liver biopsy in regard of important histological information on late graft function or guide for modification of immunosuppressant (2, 7, 9-15). And the finding of normal or near-normal graft histology in a protocol biopsy may regarded as an important baseline assessment for recpients who may be tolerant to immunosuppression weaning (2, 16-18).

Not surprisingly, histological abnormalities are more frequently seen when biopsies are taken to investigate the cause of abnormal graft function. Besides, abnormal graft histology has also been observed in long-term protocol biopsies from 5% to 85% of adults and 32% to 97% of children who are clinically well with normal liver biochemistry (2, 6, 13, 19-22). However, little is known about the results and clinical importance of late protocol biopsy after LT yet, especially for graft fibrosis and fatty change even though increasing concern and studies.

Therefore, we performed this study and the aims of this were: 1) to evaluate the graft fibrosis and fatty change based on late protocol biopsy after LT (>1 year), and 2) to investigate possible relationships between various peri-transplant clinical data and graft fibrosis or fatty change.

Patients and Methods

Patients

We retrospectively reviewed the recipients who underwent late protocol liver biopsy (> 1 year after LT) at Seoul National University Hospital between August 2010 and August 2012. As our policy, protocol liver biopsies after LT was initially done at around postoperative day 7 and continued at 1 year, 3 years, 5 years in non-HCV related patients. In case of HCV related patients, protocol liver biopsies after LT was done at about postoperative day 7, at 3 months, 6 months, 1 year and then annually.

Total 174 late protocol liver biopsies were done in 131 adult patients. They underwent LT at from April 1999 to August 2011. We excluded patients who underwent event-driven biopsy or if they were less than 16 years of age. The characteristics of the patients, including the gender, age at LT, age at biopsy, interval time of biopsy from LT, body mass index (BMI) at biopsy, and primary liver disease are shown in Table 1.

Materials and Methods

From medical records, we noted past history (such as alcohol or smoking), underlying diseases such as DM or dyslipidemia, laboratory data including liver function tests and post-LT clinical course and so on. Sono-guided fine needle aspiration liver biopsies were done by specialized radiologists. Hematoxylin and eosin and Masson trichrome stains were performed, and the pathologic review was done for the presence and severity of graft fibrosis and

fatty change by two specific hepatopathologists.

The staging of graft fibrosis was done based on the fibrosis scoring from the METAVIR system and severity of fibrosis was evaluated using a four-point scale: F0 = no fibrosis, F1 = fibrous portal expansion (mild fibrosis), F2 = few bridges or septa (moderate fibrosis), F3 = numerous bridges or septa (severe fibrosis) F4 = cirrhosis (23). The presence and severity of graft fatty change were evaluated using a steatosis grading and scoring system devised by the Pathology Committee of the NASH Clinical Research Network: S0 = normal to minimal fatty change (< 5%), S1 = mild fatty change (5 - 33%), S2 = moderate fatty change (34 - 66%), S3 = marked or severe fatty change (> 66%) (23-25).

To investigate possible relationships between various peri-transplant clinical data and graft fibrosis or fatty change, variables including recipient and donor factors at LT, parameters of post-LT conditions and about immunosuppressant, recipient factors at biopsy, pathologic findings were analyzed. And for analysis, cases of biopsy were divided into two groups by followings, four times respectively: 1) presence of graft fibrosis or not, 2) normal to mild fibrosis and significant (moderate and more) fibrosis, 3) presence of fatty change or not, 4) normal to mild fatty change and significant (moderate to severe) fatty change.

Statistical Analysis

Data were analyzed by SPSS version 19.0. Continuous variables were compared with Student's *t* test and categorical variables were compared using

the Pearson's Chi-square test or the Fisher's exact test if suspected cell frequency was less than 5 or linear by linear association if the variable has two more than categories. For multivariate analysis of the association between various peri-transplant clinical data and graft fibrosis or fatty change, we used the Linear Logistic regression analyses. All p values are two-sided and $p < 0.05$ was considered statistically significant.

Results

Graft Fibrosis

Graft Fibrosis in Late Protocol Biopsy after LT

Among the 174 late protocol biopsies after LT, total 41 cases (23.6%) showed graft fibrosis and mild, moderate, severe fibrosis were in 12.6% (n = 22), 6.9% (n = 12), 4.0% (n = 7) based on METAVIR scoring system respectively (Table 2). Among the 41 cases of fibrosis, significant (moderate and more) fibrosis was 46.3% (n = 19/41).

Univariate Analysis of Clinically Related Factors for Graft Fibrosis in Late Protocol Biopsy

We analyzed the different clinical parameters between two groups divided by presence of graft fibrosis. Table 3 showed the results of univariate analysis of clinically related factors for graft fibrosis in late protocol biopsy. DM at biopsy (25.6% vs. 46.3%, $p = 0.011$), mean fasting blood sugar (FBS) during 6 months before biopsy (107.80 ± 28.46 mg/dL vs. 118.62 ± 33.65 mg/dL; $p = 0.043$), high mean FBS (≥ 126 mg/dL) during 6 months before biopsy regardless of treatment of DM (9.8% vs. 31.7%, $p = 0.001$) showed significant difference between the groups without fibrosis and the other group with fibrosis, respectively. At the time of biopsy, clinically abnormal aspartate transaminase (AST) or alanine transaminase (ALT) was 12.8% vs. 31.7% in two groups ($p = 0.005$), and clinically abnormal alkaline phosphatase (ALP) or gamma-glutamyl transpeptidase (GGT) was 12.0% vs. 26.8% in two

groups (p = 0.022), respectively. In immunohistochemistry of pathologic findings, positive ductular reaction and bile duct damage in cytokeratin-19 (CK-19) showed significant differences between two groups. Positive ductualr reaction in CK-19 was 9.8% (n = 10/102) vs. 37.8% (n = 14/37) in group without vs. with fibrosis, respectively (p < 0.001). And, positive bile duct damage in CK-19 was 5.9% (n = 6/102) vs. 18.9% (n = 7/37) in group without vs. with fibrosis, respectively (p = 0.020). (Table 3)

Multivariate Analysis Based on Univariate Analysis of Clinically Related Factors for Graft Fibrosis in Late Protocol Biopsy

The above factors found to be statistically significant on univariate analysis and variable of post-LT biliary procedure were examined by multivariate analysis. By means of linear logistic regression, we found that high mean FBS (≥ 126 mg/dL) during 6 months before biopsy regardless of treatment of DM (risk ratio 7.260, p = 0.001) and positive ductular reaction in CK-19 (risk ratio 7.931, p < 0.001) were strong significant factors for graft fibrosis after 1 year from LT in our study (Table 4).

Univariate Analysis of Clinically Related Factors for Significant (Moderate and More) Graft Fibrosis in Late Protocol Biopsy

We analyzed the different clinical parameters between two groups of patients without and with significant (moderate and more) fibrosis. Table 5 showed the results of univariate analysis of these. Post-LT biliary procedure (16.1 vs. 42.1%, p = 0.006), high mean FBS (≥ 126 mg/dL) during 6 months before

biopsy regardless of treatment of DM (12.9% vs. 31.6%, $p = 0.031$), clinically abnormal ALP or GGT (12.9% vs. 36.8%, $p = 0.007$) showed significant differences between the group of normal to mild fibrosis and the other group of moderate and more graft fibrosis, respectively. In immunohistochemistry of pathologic findings, positive ductular reaction in CK-19 was 12.3% (n = 15/122) vs. 52.9% (n = 9/17) in group without vs. with significant fibrosis ($p < 0.001$). And, positive bile duct damage in CK-19 was 5.7% (n = 7/122) vs. 35.3% (n = 6/17) between two groups, each ($p < 0.001$) (Table 5).

Multivariate Analysis Based on Univariate Analysis of Clinically Related Factors for Significant Graft Fibrosis in Late Protocol Biopsy

The above factors found to be statistically significant on univariate analysis were examined by multivariate analysis. By means of linear logistic regression, positive ductular reaction in CK-19 (risk ratio 20.335, $p < 0.001$) and positive bile duct damage in CK-19 (risk ratio 17.351, $p = 0.001$) were strongly significant factors for significant graft fibrosis after 1 year from LT in our study (Table 6).

Graft Fatty Change

Graft Fatty Change in Late Protocol Biopsy after LT

Among the 174 late protocol biopsies after LT, total 44 cases (25.3%) showed graft fatty change and mild, moderate, severe fatty changes were in 17.2% (n = 30), 5.2% (n = 9) and 2.9% (n = 5) based on the steatosis grading and scoring system devised by the Pathology Committee of the NASH Clinical

Research Network respectively (Table 7). Among the 44 cases of fatty change, significant (moderate and severe) fatty change was 31.8% (n = 14/44).

Univariate Analysis of Clinically Related Factors for Graft Fatty change in Late Protocol Biopsy

We analyzed the different clinical parameters between two groups divided by presence of graft fatty change. Table 8 showed the results of univariate analysis of clinically related factors for graft fatty change in late protocol biopsy. Between two groups of patients without and with fatty change, recipient's age at LT (52.41 ± 8.56 years vs. 49.45 ± 7.73 years, p = 0.044), history of hepatocellular carcinoma (HCC) (55.4% vs. 36.4%, p = 0.036), positivity of HCV ribonucleic acid (RNA) at the time of biopsy (10.8% vs. 0.0%, p = 0.022), and bile duct damage in CK-19 (12.4% vs. 0.0%, p = 0.038) showed statistically significant and negative correlation with presence of fatty change. Model for End-Stage Liver Disease score (18.84 ± 9.77 vs. 22.62 ± 10.43, p = 0.043), alcohol history before LT (6.9% vs. 25.0%, p = 0.004), BMI at biopsy (23.29 ± 2.58 kg/m^2 vs. 25.54 ± 2.56 kg/m^2, p < 0.001), mean GGT at biopsy (74.48 ± 201.52 IU/L vs. 34.41 ± 40.15 IU/L, p = 0.034) showed significant differences in two groups. Additionally, male sex showed more in the group of fatty change (65.4% vs. 81.8%, p = 0.057), but not statistically significant. However, clinically, age lower than 50 years at LT (35.4% vs. 40.9%, p = 0.588), high MELD score (≥ 15 points) (57.7% vs. 71.8%, p = 0.131) and high GGT (> 35 IU/L) at biopsy (25.4% vs. 25.0%, p = 1.000) did not show significant differences, so we ruled out these three

parameters in multivariate analysis (Table 8).

Multivariate Analysis Based on Univariate Analysis of Clinically Related Factors for Graft Fatty Changes in Late Protocol Biopsy

Among the above factors found to be statistically significant on univariate analysis, male sex, history of HCC, alcohol history before LT, BMI ≥ 25 kg/m^2 at biopsy, positivity of HCV RNA at biopsy and bile duct damage in CK-19 were examined by multivariate analysis. By means of linear logistic regression, male sex (risk ratio 3.448, $p = 0.037$), alcohol history before LT (risk ratio 5.755, $p = 0.013$) and BMI ≥ 25 kg/m^2 at biopsy (risk ratio 2.926, $p = 0.020$) were clinically related factors for graft fatty change after 1 year from LT in our study (Table 9).

Univariate Analysis of Clinically Related Factors for Significant (Moderate and More) Fatty Change in Late Protocol Biopsy

We analyzed the different clinical parameters between two groups of patients without and with significant (moderate and severe) fatty change. Table 10 showed the results of univariate analysis of these. History of HCC (53.1% vs. 21.4%, $p = 0.027$) was negatively correlated with significant fatty change. Alcohol history before LT (10.0% vs. 28.6%, $p = 0.060$) and BMI ≥ 25 kg/m^2 at biopsy (31.9% vs. 64.3%, $p = 0.014$) showed significant differences in two groups (Table 10).

Multivariate Analysis Based on Univariate Analysis of Clinically Related

Factors for Significant Fatty Changes in Late Protocol Biopsy

We underwent multivariate analysis with the parameters of history of HCC, alcohol history before LT, BMI ≥ 25 kg/m^2 at biopsy, and then, BMI ≥ 25 kg/m^2 at biopsy showed significant difference (risk ratio 3.481, $p = 0.037$) in two groups (Table 11).

Discussion

Most of the main complications that occur during the early post-LT period can also be seen in late post-transplant biopsies (> 1 year after LT) (26). Among them, in this study, we focused to graft fibrosis and fatty change based on late protocol biopsy. This study demonstrated that a significant portion of late protocol biopsy after LT displayed graft fibrosis (23.6 %) and fatty change (25.3 %). Furthermore, even though based on protocol biopsy rather than event-driven biopsy, significant (moderate and more) fibrosis and fatty change of graft showed in 46.3 % and 31.8% of patients with histological fibrosis and fatty change (excluding minimal changes in both), respectively.

Graft Fibrosis in late protocol biopsy after LT

There is a concept that otherwise unexplained inflammatory changes in late post-transplant biopsies are likely to have an alloimmune basis, and have the potential to progress to graft fibrosis or cirrhosis (2). The fact that these changes are frequently seen in protocol biopsies from patients who are clinically well with normal liver function tests suggests that protocol biopsies have an important role in identifying subclinical graft dysfunction in patients surviving long-term following LT (2, 7). Particularly, in HCV related recipient, histological abnormalities are often present in protocol biopsies from HCV-positive patients who are clinically well, with apparently normal graft function (27, 28) and the changes seen in these specimens may have implications for prognosis and treatment (2).

Until about a decade ago, fibrosis was considered a progressive process that could be halted, but would not regress. However, hepatic fibrosis is currently viewed as a dynamic process that can progress or regress and even the excess fibrous tissue of cirrhotic livers has been found to sometimes regress over time (1). There is no doubt that graft fibrosis is very important finding and related with post-LT outcome and long-term graft survival regardless of the underlying disease and main cause of fibrosis including HCV recurrence. Therefore, to evaluate the pathophysiology and clinical correlation of graft fibrosis is important and it can be helpful to improve or reverse the dynamic process of fibrotic change of liver graft.

Scheenstra et al (13) reported the graft fibrosis after 10 years following pediatric LT. In that study, fibrosis was strongly related to transplant-related factors such as prolonged cold ischemic time, young age at the time of transplantation, high ratio of donor to recipient age, and the use of partial grafts. Although the function of the grafts with development of fibrosis still seemed adequate at 10 years, they did realize, however, that the progression of fibrosis toward cirrhosis could endanger graft function and graft survival in the longer term (10 years after LT). In another study, Rifai et al reported that long-term development of fibrosis seems to be associated with the donor age, independently of HCV status (29, 30).

In our study, in univariate analysis, there were no significant variables in recipient or donor factors at LT including recipient and donor age, ratio of donor to recipient age and partial graft. Also, post-LT conditions and variables about immunosuppressant did not show significant differences in two groups

according to presence of fibrosis. But, we included the variable of post-LT biliary procedure such as percutaneous transhepatic biliary drainage or endoscopic retrograde cholangiopancreatogra at multivariate analysis because it was clinically suspected significant factor and the *p* value closed to the 0.05 (p = 0.054). Through multivariate analysis with significant variables in univariate analysis, high mean FBS (≥ 126 mg/dL) during 6 months before biopsy regardless of treatment of DM (risk ratio 7.260, p = 0.001) and positive ductular reaction in CK-19 (risk ratio 7.931, p < 0.001) were significantly related factors for graft fibrosis after 1 year from LT.

For significant fibrosis, positive ductular reaction in CK-19 (risk ratio 20.335, p < 0.001) and positive bile duct damage in CK-19 (risk ratio 17.351, p = 0.001) were strongly related factors for moderate and more graft fibrosis after 1 year from LTs in multivariate analysis.

Based on these results, we have to concern about the late graft fibrosis more in patients with high FBS regardless of treatment of DM (that is, uncontrolled DM) and positive ductular reaction in CK-19 (clinically, it can be related with recurred hepatitis or cholangitis). Therefore, if we can well control the DM and prevent or reduce the ductular reaction (such as prevention or treatment of cholangitis, recurrence of viral hepatitis, etc.), we can prevent or reduce the late graft fibrosis and improve the long-term outcome of recipient. In significant graft fibrosis, as well-known, if we can reduce the bile duct damage and the ductular reaction, we can reduce the significant fibrosis in late graft. Further more, bile duct damage and probably ductular reaction in CK-19 are related with biliary problem or complication. Therefore we can prevent

or reduce the graft fibrosis by efforts decresing biliary complications. Howerever, we do not know much about the ductular reaction in CK-19 in graft yet (31), so more study will be needed in larger group in the future. Additionally, there is a possibility that the graft fibrosis reversely affected as the cause of the positive bile duct damage or ductulr reaction in CK-19 of immunohistochemistry in pathology, not a result.

In case of DM, there is no report or evidence of DM directly related with hepatic fibrosis yet, especially in transplant setting. However, only recently, there were a few studies and opinions about the association between DM and chronic liver disease or cirrhosis, even though in according to the concurrent NAFLD and not in transplant setting (32, 33). Papatheodoridis et al. (2006) showed that more severe fibrosis and particularly presence of cirrhosis were found to be independently associated with presence of DM in patients with chronic hepatitis B or C in non-transplant setting (34). However, the relationship of DM with the severity of liver disease has not been adequately clarified. And, althogh DM is present more frequently in patients with cirrhosis or even those with HCC in several studies (34-36), it is unclear whether the presence of DM precedes and accelerates or whether it follows and results from the progression of chronic liver disease. NAFLD has been known to associated with Type 2 DM and hepatic fibrosis in many studies (37) on the contrary to our study that showed no significant correlation of graft fatty change with DM or fibrosis. Petit et al. (2011) reported that they comfirmed the influence of PNPLA3 rs738409 polymorphism on the severity of steatosis and liver fibrosis in a population of patients suffering from type 2

DM (37). However, nothing is conclusive and further study is needed, especially in LT setting.

Graft Fatty change in late protocol biopsy after LT

NAFLD is a common cause of chronic liver disease and is strongly associated with obesity and metabolic syndrome (38). However, the long-term outcome for NAFLD patients remains controversial. Although some studies suggest increased overall mortality and liver-specific mortality in patients with NAFLD, others do not (39-42).

Recently, Younossi et al. reported that patients with NAFLD accompanying with metabolic syndrome are at increased risk for mortality, primarily from cardiac death and possibly liver-related death (38).

In setting of LT, there are a few of studies about fatty change or NAFLD. Moreover, there is no study about long-term outcome after LT related with post-LT NAFLD or metabolic syndrome yet. Naturally, we do not know exactly about the importance of post-LT NAFLD and impact of fatty change on long-term outcome of recipient, even in moderate and more degree. However, by inference from relationship of general population with NAFLD and their long-term outcome in non-LT setting, post-LT fatty change may influence long-term outcome in recipients.

Some studies have shown that recurrence of NAFLD is common after LT, but, the incidence, risk factors, and natural history of de novo NAFLD following LT have not been well elucidated (43-46). Seo et al. retrospectively analyzed and reported that 18% (n = 12) among the 68 recipients was

histologically diagnosed. In this study, related risk factors were an increase in BMI as a positive factor and the use of angiotensin converting enzyme inhibitor as a negative factor (5). However, rapid weight gain, DM, hyperlipidemia, hypertension, and consequent metabolic syndrome are more common post-LT than in the general population; therefore, the risk of developing NAFLD in this population may increase (4, 5).

In our study, as previously mentioned, graft fatty change based on late protocol biopsy was 25.3 % and significant fatty change was 31.8%. With multivariate analysis, male sex (risk ratio 3.448, $p = 0.037$), alcohol history before LT (risk ratio 5.755, $p = 0.013$), obesity (BMI $\geq$ 25 kg/m^2) at the time of biopsy (risk ratio 2.926, $p = 0.020$) were clinically related factors for graft fatty change after 1 year from LT. In respect of significant fatty change, only obesity (BMI $\geq$ 25 kg/m^2) at the time of biopsy (risk ratio 3.481, $p = 0.037$) was significant related factor.

As it is well-known, fatty change of liver is reversible throughout life-style modification such as body weight reduction, diet control, exercise etc. Therefore, according to our study, body weight control and avoiding obesity at post-LT can prevent or improve late graft fatty change. In especially the male recipients having previous alcohol history before LT, there is an increased risk of fatty liver after LT, so we have to more concern about possibility of late graft fatty change in these patients. Particularly, in patients with obesity (BMI $\geq$ 25 kg/m^2) at post-LT, there is a high risk of significant graft fatty change (risk ratio 3.481). These graft fatty change especially in significant degree may be related with metabolic syndrome and long-term

graft survival and outcome, even though no one knows exactly yet. More concern and further study in this field is needed in the future, also.

This study had some limitations, especially with respect to the relatively small sample size and follow-up period and diverse interval time of biopsy from LT. Therefore, we are planning to continue this study and serial protocol biopsies at specific time points such as post-LT 1 year, 3 year, 5 year and 10 year henceforward.

Even though recent several evidences about usefulness of late protocol biopsy after LT, the use of protocol liver biopsy, except in the event of HCV infection, has been largely ababdoned and still debated (2, 19). Therefore, well-designed further studies in this field should be and needed.

In conclusion, the graft fibrosis and fatty change based on late protocol biopsy after LT comprised a considerable portion. In this study, high mean FBS (≥ 126 mg/dL) during 6 months before biopsy regardless of treatment of DM and positive ductular reaction in CK-19 were closely related to the graft fibrosis, and male sex, alcohol history before LT and obesity (BMI ≥ 25 kg/m^2) at biopsy were related to graft fatty change. We can apply these results based on late protocol biopsy to management for recipients and that may be a good influence to future long-term outcome of the patients.

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Table 1. Characteristics of Cases of Late Protocol Liver Biopsies (n = 174)

Gender (male: female)	121: 53 (69.5%: 30.5%)
Mean age at LT (year)	51.66 ± 8.44 (range, 17 - 69)
Mean age at biopsy (year)	54.59 ± 8.47 (range, 19 - 73)
Mean interval time of biopsy from LT (month)	38.58 ± 30.88 (range, 12 - 151)
Mean BMI at biopsy (kg/m^2)	23.86 ± 2.75 (range, 18.6 - 33.1)
LDLT : DDLT	113: 61 (64.9%: 35.1%)
Primary liver diseases	
Hepatitis B related liver cirrhosis	122 (70.1%)
Hepatitis C related liver cirrhosis	26 (14.9%)
Combined with HCC	88 (50.6%)

Abbreviation: LT, Liver transplantation; BMI, Body mass index; LDLT, Living donor LT; DDLT, Deceased donor LT, HCC, Hepatocellular carcinoma.

Table 2. Graft Fibrosis Based on METAVIR scoring

METAVIR scoring	Cases (n)	Percentage
Total (Point)	174	100.0%
No fibrosis (0)	133	76.4%
Fibrosis	41	23.6%
Mild (1)	22	12.6%
Moderate (2)	12	6.9%
Severe (3)	7	4.0%
Cirrhosis (4)	0	0.0%

Table 3. Univariate Analysis of Clinically Related Factors for Graft Fibrosis in Late Protocol Biopsy

Variables of Clinically Related Factors for Graft Fibrosis	**No Fibrosis N = 133**	**Fibrosis N = 41**	***P* values**
RECIPIENT FACTORS at LT			
Recipient age at LT (year)	51.76 ± 8.52	51.34 ± 8.25	0.782
Sex (male)	90 (67.7%)	31 (75.6%)	0.334
Underlying disease			
Hepatitis B related	96 (72.2%)	26 (63.4%)	0.284
Hepatitis C related	17 (12.85%)	9 (22.0%)	0.150
Hepatocellular carcinoma	69 (51.9%)	19 (46.3%)	0.535
MELD score	17.62 ± 11.59	15.34 ± 11.47	0.271
Pre-LT alcohol history	16 (12.0%)	4 (9.8%)	0.787
Pre-LT smoking history	10 (7.5%)	5 (12.2%)	0.351
DM at LT	38 (28.6%)	18 (43.90%)	0.066
In-hospital stay (day)	18.18 ± 11.09	24.59 ± 34.54	0.249
DONOR FACTORS at LT			
Donor age at LT (year)	31.36 ± 11.72	28.56 ± 14.08	0.232
Deceased donor LT	44 (33.1%)	17 (41.5%)	0.325
Ratio of donor to recipient age	0.63 ± 0.25	0.65 ± 0.28	0.593
Graft to recipient weight ratio	1.48 ± 0.60	1.64 ± 0.69	0.161
Graft fatty change	31 (23.3%)	7 (17.1%)	0.162

POST-LT CONDITIONS			
HBV recurrence (or de novo)			
Positive HBsAg	7/96 (7.3%)	5/28 (17.9%)	0.096
HCV recurrence			
Positive HCV RNA	15/17 (88.2%)	9/9 (100%)	0.529
Post-LT dyslipidemia	4 (3.0%)	1 (2.4%)	0.849
Post-LT complication[1]	39 (29.3%)	10 (24.4%)	0.539
Post-LT biliary procedure[2]	21 (15.8%)	12 (29.3%)	0.054
Post-LT portal vein intervention	5 (3.8%)	3 (7.3%)	0.394
RECIPIENT FACTORS at Bx.			
Recipient age at Bx. (year)	54.63 ± 8.42	54.44 ± 8.70	0.899
BMI at Bx. (kg/m^2)	24.02 ± 2.84	23.35 ± 2.39	0.174
Hypertension at Bx.	35 (26.3%)	12 (29.3%)	0.710
DM at Bx.	**34 (25.6%)**	**19 (46.3%)**	**0.011**
Mean FBS during 6 months before Bx. (mg/dL)	**107.80 ± 28.46**	**118.62 ± 33.65**	**0.043**
≥ 126 mg/dL	**13 (9.8%)**	**13 (31.7%)**	**0.001**
Interval time of Bx. from LT (month)	37.41 ± 31.54	42.39 ± 28.68	0.368
Laboratory result at Bx.			
Cholesterol (mg/dL)	160.54 ± 55.13	152.80 ± 35.80	0.400
Total bilirubin (mg/dL)	1.68 ± 4.66	1.45 ± 1.21	0.755
AST (IU/L)	28.65 ± 43.04	50.41 ± 115.22	0.243

ALT (IU/L)	33.02 ± 74.91	68.15 ± 188.32	0.250
ALP (IU/L)	92.59 ± 116.49	100.54 ± 51.27	0.673
GGT (IU/L)	61.85 ± 185.50	72.46 ± 142.74	0.737
Platelet ($10^3/\mu\ell$)	146.31 ± 51.55	140.98 ± 69.67	0.652
PT INR	0.94 ± 0.22	0.97 ± 0.25	0.463
Positive HBV DNA	9 (6.8%)	4 (9.8%)	0.508
Positive HCV RNA	9 (6.8%)	5 (12.2%)	0.264
Abnormal AST or ALT at Bx.	**17 (12.8%)**	**13 (31.7%)**	**0.005**
Abnormal ALP or GGT at Bx.	**16 (12.0%)**	**11 (26.8%)**	**0.022**
PATHOLOGIC FINDINGS			
C4d	11/79 (13.8%)	6/30 (20.0%)	0.435
CK-19			
Ductular reaction	**10/102(9.8%)**	**14/37(37.8%)**	**<0.001**
Bile duct damage	**6/102(5.9%)**	**7/37(18.9%)**	**0.020**
Fatty change on Bx.			
Positive fatty change	35 (26.3%)	12 (29.3%)	0.710
Grade 2 or 3 fatty change	9 (6.8%)	5 (12.2%)	0.264
IMMUNOSUPPRESSANT			
Steroid withdrawal after LT			
Using period after LT (month)	6.72 ± 7.66	6.95 ± 7.36	0.864
Withdrawal within 3 months	109/131(83.2%)	37/41 (90.2%)	0.272
Withdrawal within 6 months	55 (41.4%)	19 (46.3%)	0.482
Steroid use on Bx.	13 (9.8%)	6 (14.6%)	0.383

Kind of main IS			
Main initial IS (Cyclosporine)	6 (4.5%)	2 (4.9%)	1.000
Main IS at Bx. (Cyclosporine)	10 (7.5%)	4 (9.8%)	0.534
Main IS level (pre-Bx. 3 months)			
Lower than therapeutic range	61/132 (46.2%)	18/41 (43.9%)	0.932

Abbreviation: LT, Liver transplantation; MELD, Model for End-Stage Liver Disease; DM, diabetes mellitus; HBV, Hepatitis B virus; HCV, Hepatitis C virus; RNA, Ribonucleic acid; Bx., Biopsy; BMI, Body mass index; FBS, Fasting blood sugar; AST, Aspartate transaminase; ALT, Alanine transaminase; ALP, Alkaline phosphatase; GGT, Gamma-glutamyl transpeptidase; PT INR, Prothrombin time International normalized ratio; DNA, Deoxyribonucleic acid; C4d, Polyclonal complement fragment 4d; CK-19, Cytokeratin-19; IS, Immunosuppressant.

[1] Clavien classification grade IIIa and more

[2] Percutaneous transhepatic biliary drainage or Endoscopic retrograde cholangiopancreatography

Table 4. Multivariate Analysis Based on Univariate Analysis of Clinically Related Factors for Graft Fibrosis in Late Protocol Biopsy

Variables	***P* values**	**Risk ratio**	**95% CI**
Post-LT biliary procedure[1]	0.338	1.672	0.584 - 4.785
Mean FBS ≥ 126 mg/dL during 6 months before Bx.	**0.001**	**7.260**	**2.293 - 22.986**
Abnormal AST or ALT at Bx.	0.161	2.406	0.705 - 8.213
Abnormal ALP or GGT at Bx.	0.541	0.657	0.171 - 2.523
CK-19, ductular reaction	**< 0.001**	**7.931**	**2.768 - 22.723**
CK-19, bile duct damage	0.273	2.326	0.515- 10.516

Abbreviation: LT, Liver transplantation; FBS, Fasting blood sugar; Bx., Biopsy; AST, Aspartate transaminase; ALT, Alanine transaminase; ALP, Alkaline phosphatase; GGT, Gamma-glutamyl transpeptidase; CK-19, Cytokeratin-19

[1] Percutaneous transhepatic biliary drainage or Endoscopic retrograde cholangiopancreatography

Table 5. Univariate Analysis of Clinically Related Factors for Significant Graft Fibrosis (Moderate and More) in Late Protocol Biopsy

Variables of Clinically Related Factors for Significant Graft Fibrosis	**Fibrosis 0-1 N = 155**	**Fibrosis 2-3 N = 19**	***P* values**
RECIPIENT FACTORS at LT			
Recipient age at LT (year)	52.08 ± 8.22	48.21 ± 9.60	0.059
Sex (male)	106 (68.4%)	15 (78.9%)	0.345
Underlying disease			
Hepatitis B related	111 (71.6%)	11 (57.9%)	0.218
Hepatitis C related	21 (13.5%)	5 (26.3%)	0.141
Hepatocellular carcinoma	80 (51.6%)	8 (42.1%)	0.434
MELD score	17.25 ± 11.45	15.74 ± 12.81	0.592
Pre-LT alcohol history	17 (11.0%)	3 (15.8%)	0.534
Pre-LT smoking history	13 (8.4%)	2 (10.5%)	0.670
DM at LT	49 (31.6%)	7 (36.8%)	0.645
In-hospital stay (day)	19.90 ± 18.63	18. ± 25.49	0.689
DONOR FACTORS at LT			
Donor age at LT (year)	31.08 ± 11.85	27.71 ± 15.70	0.287
Deceased donor LT	52 (33.5%)	9 (47.4%)	0.233
Ratio of donor to recipient age	0.62 ± 0.24	0.78 ± 0.33	0.105
Graft to recipient weight ratio	1.52 ± 0.63	1.57 ± 0.59	0.749
Graft fatty change	35 (22.6%)	3 (15.8%)	0.091

POST-LT CONDITIONS			
HBV recurrence (or de novo)			
Positive HBsAg	10/112 (8.9%)	2/12 (16.7%)	0.327
HCV recurrence			
Positive HCV RNA	19/21 (90.5%)	5/5 (100%)	1.000
Post-LT dyslipidemia	5 (3.2%)	0 (0.0%)	1.000
Post-LT complication1	45 (29.0%)	4 (21.1%)	0.594
Post-LT biliary procedure[2]	**25 (16.1%)**	**8 (42.1%)**	**0.006**
Post-LT portal vein intervention	7 (4.5%)	1 (5.3%)	1.000
RECIPIENT FACTORS at Bx			
Recipient age at Bx. (year)	55.01 ± 8.19	51.16 ± 10.07	0.061
BMI at Bx. (kg/m2)	23.93 ± 2.79	23.30 ± 2.35	0.343
Hypertension at Bx.	41 (26.5%)	6 (31.6%)	0.635
DM at Bx.	44 (28.4%)	9 (47.4%)	0.090
Mean FBS during 6 months before Bx. (mg/dL)	108.15 ± 27.58	128.25 ± 42.14	0.056
≥ 126 mg/dL	**20 (12.9%)**	**6 (31.6%)**	**0.031**
Interval time of Bx. from LT (month)	38.05 ± 31.23	42.89 ± 28.27	0.520
Laboratory result at Bx.			
Cholesterol (mg/dL)	160.28 ± 52.54	145.95 ± 37.78	0.251
Total bilirubin (mg/dL)	1.61 ± 4.33	1.78 ± 1.55	0.863
AST (IU/L)	29.37 ± 42.37	69.79 ± 164.37	0.300

ALT (IU/L)	35.85 ± 77.74	85.74 ± 260.89	0.418
ALP (IU/L)	92.03 ± 109.19	114.32 ± 54.78	0.383
GGT (IU/L)	59.45 ± 173.32	104.32 ± 97.28	0.296
Platelet (103/$\mu\ell$)	144.96 ± 52.32	145.79 ± 83.11	0.967
PT INR	0.94 ± 0.21	1.01 ± 0.29	0.204
Positive HBV DNA	12 (7.7%)	1 (5.3%)	1.000
Positive HCV RNA	11 (7.1%)	3 (15.8%)	0.184
Abnormal AST or ALT at Bx.	24 (15.5%)	6 (31.6%)	0.080
Abnormal ALP or GGT at Bx.	**20 (12.9%)**	**7 (36.8%)**	**0.007**
PATHOLOGIC FINDINGS			
C4d	13/96 (13.5%)	4/13 (30.8%)	0.118
CK-19			
Ductular reaction	**15/122 (12.3%)**	**9/17(52.9%)**	**<0.001**
Bile duct damage	**7/122 (5.7%)**	**6/17 (35.3%)**	**<0.001**
Fatty change on Bx.			
Positive fatty change	45 (29.0%)	2 (10.5%)	0.105
Grade 2 or 3 fatty change	12 (7.7%)	2 (10.5%)	0.653
IMMUNOSUPPRESSANT			
Steroid withdrawal after LT			
Using period after LT (month)	6.86 ± 7.88	6.11 ± 4.46	0.685
Withdrawal within 3 months	129 (84.3%)	17 (89.5%)	0.554
Withdrawal within 6 months	64 (41.3%)	10 (52.6%)	0.588
Steroid use on Bx.	17 (11.0%)	2 (10.5%)	1.000

Kind of main IS			
Main initial IS (Cyclosporine)	8 (5.2%)	0 (0.0%)	0.601
Main IS at Bx. (Cyclosporine)	13 (8.4%)	1 (5.3%)	0.437
Main IS level (pre-Bx. 3months)			
Lower than therapeutic range	69 (44.8%)	10 (52.6%)	0.737

Abbreviation: LT, Liver transplantation; MELD, Model for End-Stage Liver Disease; DM, diabetes mellitus; HBV, Hepatitis B virus; HCV, Hepatitis C virus; RNA, Ribonucleic acid; Bx., Biopsy; BMI, Body mass index; FBS, Fasting blood sugar; AST, Aspartate transaminase; ALT, Alanine transaminase; ALP, Alkaline phosphatase; GGT, Gamma-glutamyl transpeptidase; PT INR, Prothrombin time International normalized ratio; DNA, Deoxyribonucleic acid; C4d, Polyclonal complement fragment 4d; CK-19, Cytokeratin-19; IS, Immunosuppressant.

[1] Clavien classification grade IIIa and more

[2] Percutaneous transhepatic biliary drainage or Endoscopic retrograde cholangiopancreatography

Table 6. Multivariate Analysis Based on Univariate Analysis of Clinically Related Factors for Significant Fibrosis in Late Protocol Biopsy

Variables	***P* values**	**Risk ratio**	**95% CI**
Mean FBS ≥ 126 mg/dL during 6 months before Bx.	0.116	3.377	0.739 - 15.434
Post-LT biliary procedure[1]	0.633	1.416	0.339 - 5.917
Abnormal ALP or GGT at Bx.	0.858	1.141	0.270 - 4.815
CK-19, ductular reaction	**< 0.001**	**20.335**	**4.616 - 89.579**
CK-19, bile duct damage	**0.001**	**17.351**	**3.003 - 100.248**

Abbreviation: FBS, Fasting blood sugar; Bx., Biopsy; LT, Liver transplantation; ALP, Alkaline phosphatase; GGT, Gamma-glutamyl transpeptidase; CK-19, Cytokeratin-19

[1] Percutaneous transhepatic biliary drainage or Endoscopic retrograde cholangiopancreatography

Table 7. Fatty Change Based on the Steatosis Grading and Scoring System devised by the Pathology Committee of the NASH Clinical Research Network

Steatosis	**Cases (n)**	**Percentage**
Total (Point)	174	100.0%
Normal to minimal fatty change (0)	130	74.7%
Fatty change	44	25.3%
Mild (1)	30	17.2%
Moderate (2)	9	5.2%
Severe (3)	5	2.9%

Table 8. Univariate Analysis of Clinically Related Factors for Graft Fatty change in Late Protocol Biopsy

Variables of Clinically Related Factors for Fatty Change	**No Fatty Change N = 130**	**Fatty Change N = 44**	***P* values**
RECIPIENT FACTORS at LT			
Recipient age at LT (year)*	**52.41 ± 8.56**	**49.45 ± 7.73**	**0.044**
Age < 50 years	35.4%	40.9%	0.588
Sex (male)	85 (65.4%)	36 (81.8%)	0.057
Underlying disease			
Hepatitis B related	91 (70.0%)	31 (70.5%)	1.000
Hepatitis C related	23 (17.7%)	4 (9.1%)	0.230
Hepatocellular carcinoma*	**72 (55.4%)**	**16 (36.4%)**	**0.036**
MELD score	**18.84 ± 9.77**	**22.62 ± 10.43**	**0.043**
≥ 15	64 (57.7%)	28 (71.8%)	0.131
Pre-LT alcohol history	**9 (6.9%)**	**11 (25.0%)**	**0.004**
Pre-LT smoking history	9 (6.9%)	6 (13.6%)	0.213
DM at LT	41 (32.3%)	15 (31.9%)	0.963
In-hospital stay (day)	19.45 ± 20.45	20.39 ± 16.19	0.784
DONOR FACTORS at LT			
Donor age at LT (year)	30.58 ± 12.38	31.13 ± 12.26	0.809
Deceased donor LT	45 (34.6%)	16 (36.4%)	0.856
Ratio of donor to recipient age	0.63 ± 0.24	0.65 ± 0.29	0.630

Graft to recipient weight ratio	1.53 ± 0.60	1.52 ± 0.70	0.961
Graft fatty change	29 (22.3%)	9 (20.5%)	0.866
POST-LT CONDITIONS			
Post-LT dyslipidemia	4 (3.1%)	1 (2.3%)	1.000
Post-LT complication[1]	33 (25.4%)	16 (36.4%)	0.178
Post-LT biliary procedure[2]	29 (22.3%)	4 (9.1%)	0.074
Post-LT portal vein intervention	7 (5.4%)	1 (2.3%)	0.681
RECIPIENT FACTORS at Bx			
Recipient age at Bx. (year)	55.16 ± 8.56	52.89 ± 8.05	0.124
BMI at Bx. (kg/m^2)	**23.29 ± 2.58**	**25.54 ± 2.56**	**<0.001**
≥ 23	**71 (54.6%)**	**35 (79.5%)**	**0.004**
≥ 25	**35 (26.9%)**	**25 (56.8%)**	**<0.001**
Hypertension at Bx.	37 (28.5%)	10 (22.7%)	0.557
DM at Bx.	37 (29.1%)	16 (34.0%)	0.532
Mean FBS during 6 months before Bx. (mg/dL)	110.23 ± 32.89	110.70 ± 19.50	0.928
≥ 126 mg/dL	18 (13.8%)	8 (18.2%)	0.472
Interval time of Bx. from LT (month)	36.57 ± 29.90	44.52 ± 33.26	0.140
Laboratory result at Bx.			
Cholesterol (mg/dL)	156.62 ± 56.89	164.91 ± 28.35	0.355
≥ 200	10(7.7%)	4 (9.1%)	0.754
Total bilirubin (mg/dL)	1.87 ± 4.730	0.90 ± 0.38	0.176

AST (IU/L)	34.51 ± 71.02	31.64 ± 56.95	0.808
ALT (IU/L)	39.58 ± 116.12	46.36 ± 103.08	0.731
ALP (IU/L)	101.29± 119.03	74.30 ± 33.05	0.140
GGT (IU/L)	**74.48 ± 201.52**	**34.41 ± 40.15**	**0.034**
> 35	33 (25.4%)	11 (25.0%)	1.000
Platelet (103/$\mu\ell$)	140.68 ± 58.44	157.95 ± 47.12	0.078
PT INR	0.95 ± 0.24	0.94 ± 0.16	0.793
Positive HBV DNA	8 (6.2%)	5 (11.4%)	0.318
Positive HCV RNA*	**14 (10.8%)**	**0 (0.0%)**	**0.022**
Abnormal AST or ALT at Bx.	20 (15.4%)	10 (22.7%)	0.259
Abnormal ALP or GGT at Bx.	23 (17.7%)	4 (9.1%)	0.230
PATHOLOGIC FINDINGS			
C4d	14/78 (17.9%)	3/31 (9.7%)	0.386
CK-19			
Ductular reaction	18/105(17.1%)	6/34 (17.6%)	1.000
Bile duct damage*	**13/105(12.4%)**	**0/34(0.0%)**	**0.038**
Fibrosis on Bx.			
Positive fibrosis	29 (22.3%)	12 (27.3%)	0.540
Grade 2 or 3 fibrosis	17 (13.1%)	2 (4.5%)	0.163
IMMUNOSUPPRESSANT			
Steroid withdrawal after LT			
Using period after LT (month)	6.70 ± 6.81	7.0 ± 9.53	0.837
Withdrawal after 3 months	109 (85.2%)	37 (84.1%)	0.812

Withdrawal after 6 months	52 (40.0%)	22 (50.0%)	0.201
Steroid use on Bx.	16 (12.3%)	3 (6.8%)	0.4095
Kind of main IS			
Main initial IS (Cyclosporine)	6 (4.6%)	2 (4.5%)	1.000
Main IS at Bx. (Cyclosporine)	9 (6.9%)	5 (11.4%)	0.360
Main IS level (pre-Bx. 3months)			
Lower than therapeutic range	61 (47.3%)	18 (40.9%)	0.387

Abbreviation: LT, Liver transplantation; MELD, Model for End-Stage Liver Disease; DM, diabetes mellitus; HBV, Hepatitis B virus; HCV, Hepatitis C virus; RNA, Ribonucleic acid; Bx., Biopsy; BMI, Body mass index; FBS, Fasting blood sugar; AST, Aspartate transaminase; ALT, Alanine transaminase; ALP, Alkaline phosphatase; GGT, Gamma-glutamyl transpeptidase; PT INR, Prothrombin time International normalized ratio; DNA, Deoxyribonucleic acid; C4d, Polyclonal complement fragment 4d; CK-19, Cytokeratin-19; IS, Immunosuppressant.

[1] Clavien classification grade IIIa and more

[2] Percutaneous transhepatic biliary drainage or Endoscopic retrograde cholangiopancreatography

* Negative relation

Table 9. Multivariate Analysis Based on Univariate Analysis of Clinically Related factors for Graft Fatty change in Late Protocol Biopsy

Variables	***P* values**	**Risk ratio**	**95% CI**
Sex (male)	**0.037**	**3.448**	**1.081 – 10.998**
Hepatocellular carcinoma	0.061	0.420	0.169 – 1.042
Pre-LT alcohol history	**0.013**	**5.755**	**1.447 – 22.883**
BMI ≥ 25 kg/m^2 at Bx.	**0.020**	**2.926**	**1.184 – 7.235**
Positive HCV RNA at Bx.	0.999	0.000	0.000 –
CK-19, bile duct damage	0.998	0.000	0.000 –

Abbreviation: LT, Liver transplantation; BMI, Body mass index; Bx., Biopsy; HCV, Hepatitis C virus; RNA, Ribonucleic acid.

Table 10. Univariate Analysis of Clinically Related Factors for Significant Fatty change (Moderate and More) in Late Protocol Biopsy

Variables of Clinically Related Factors for Significant Fatty Change	**Fatty Change 0-1 N = 160**	**Fatty Change 2-3 N = 14**	***P* values**
RECIPIENT FACTORS at LT			
Recipient age at LT (year)	52.08 ± 8.22	48.21 ± 9.60	0.059
Sex (male)	110 (68.8%)	11 (78.6%)	0.444
Underlying disease			
Hepatitis B related	114 (71.3%)	8 (57.1%)	0.269
Hepatitis C related	26 (16.3%)	0 (0.0%)	0.133
Hepatocellular carcinoma*	**85 (53.1%)**	**3 (21.4%)**	**0.027**
MELD score	17.25 ± 11.44	15.74 ± 12.81	0.592
Pre-LT alcohol history	**16 (10.0%)**	**4 (28.6%)**	**0.060**
Pre-LT smoking history	14 (8.8%)	1 (7.1%)	1.000
DM at LT	50 (31.3%)	6 (42.9%)	0.373
In-hospital stay (day)	19.90 ± 18.63	18.00 ± 25.49	0.689
DONOR FACTORS at LT			
Donor age at LT (year)	31.08 ± 11.85	27.71 ± 15.70	0.287
Deceased donor LT	57 (35.6%)	4 (28.6%)	0.773
Ratio of donor to recipient age	0.62 ± 0.24	0.78 ± 0.33	0.105
Graft to recipient weight ratio	1.52 ± 0.63	1.57 ± 0.59	0.749
Graft fatty change	35 (21.9%)	3 (21.4%)	0.787

POST-LT CONDITIONS			
Post-LT dyslipidemia	5 (3.1%)	0 (0.0%)	1.000
Post-LT complication[1]	44 (27.5%)	5 (35.7%)	0.512
Post-LT biliary procedure[2]	32 (20.0%)	1 (7.1%)	0.474
Post-LT portal vein intervention	8 (5.0%)	0 (0.0%)	1.000
RECIPIENT FACTORS at Bx			
Recipient age at Bx. (year)	55.01 ± 8.19	51.16 ± 10.07	0.061
BMI at Bx. (kg/m^2)	**23.696 ± 2.67**	**25.736 ± 3.01**	**0.007**
≥ 23	96 (60.0%)	10 (71.4%)	0.401
≥ 25	**51 (31.9%)**	**9 (64.3%)**	**0.014**
Hypertension at Bx.	43 (26.9%)	4 (28.6%)	1.000
DM at Bx.	48 (30.0%)	5 (35.7%)	0.656
Mean FBS during 6 months before Bx. (mg/dL)	110.32 ± 30.65	110.62 ± 22.38	0.972
≥ 126 mg/dL	24 (15.0%)	2 (14.3%)	1.000
Interval time of Bx. from LT (month)	38.05 ± 31.23	42.89 ± 28.27	0.520
Laboratory result at Bx.			
Cholesterol (mg/dL)	160.28 ± 52.54	145.95 ± 37.78	0.251
≥ 200	14 (8.8 %)	0 (0.0%)	0.607
Total bilirubin (mg/dL)	1.61 ± 4.33	1.78 ± 1.55	0.863
AST (IU/L)	29.37 ± 42.37	69.79 ± 164.37	0.300
ALT (IU/L)	35.85 ± 77.74	85.74 ± 260.89	0.418

ALP (IU/L)	92.03 ± 109.19	114.32 ± 54.78	0.383
GGT (IU/L)	59.45 ± 173.32	104.32 ± 197.28	0.296
Platelet (103/㎕)	144.96 ± 52.32	145.79 ± 83.11	0.967
PT INR	0.94 ± 0.21	1.01 ± 0.29	0.204
Positive HBV DNA	11 (6.9%)	2 (14.3%)	0.312
Positive HCV RNA	14 (8.8%)	0 (0.0%)	0.248
Abnormal AST or ALT at Bx.	29 (18.1%)	2 (14.3%)	1.000
Abnormal ALP or GGT at Bx.	26 (16.3%)	1 (7.1%)	0.699
PATHOLOGIC FINDINGS			
C4d	16/98 (16.3%)	1/11 (9.1%)	1.000
CK-19			
Ductular reaction	22/127(17.3%)	2/12(16.7%)	1.000
Bile duct damage	13/127(10.2%)	0/12(0.0%)	0.604
Fatty change on Bx.			
Positive fatty change	36 (22.5%)	5 (35.7%)	0.264
Grade 2 or 3 fatty change	17 (10.6%)	2 (14.3%)	0.653
IMMUNOSUPPRESSANT			
Steroid withdrawal after LT			
Using period after LT (month)	6.86 ± 7.88	6.11 ± 4.46	0.685
Withdrawal within 3 months	135 (85.4%)	11 (78.6%)	0.491
Withdrawal within 6 months	90 (42.5%)	6 (42.9%)	0.915
Steroid use on Bx.	19 (11.9%)	0 (0.0%)	0.369
Kind of main IS			

Main initial IS (Cyclosporine)	7 (4.4%)	1 (7.1%)	0.496
Main IS at Bx. (Cyclosporine)	13 (8.1%)	1 (7.1%)	0.646
Main IS level (pre-Bx. 3months)			
Lower than therapeutic range	72 (45.3%)	7 (50.0%)	0.875

Abbreviation: LT, Liver transplantation; MELD, Model for End-Stage Liver Disease; DM, diabetes mellitus; HBV, Hepatitis B virus; HCV, Hepatitis C virus; RNA, Ribonucleic acid; Bx., Biopsy; BMI, Body mass index; FBS, Fasting blood sugar; AST, Aspartate transaminase; ALT, Alanine transaminase; ALP, Alkaline phosphatase; GGT, Gamma-glutamyl transpeptidase; PT INR, Prothrombin time International normalized ratio; DNA, Deoxyribonucleic acid; C4d, Polyclonal complement fragment 4d; CK-19, Cytokeratin-19; IS, Immunosuppressant.

[1] Clavien classification grade IIIa and more

[2] Percutaneous transhepatic biliary drainage or Endoscopic retrograde cholangiopancreatography

* Negative relation

Table 11. Multivariate Analysis based on univariate analysis of Clinically Related Factors for Significant Fatty change in Late Protocol Biopsy

Variables	***P* values**	**Risk ratio**	**95% CI**
Hepatocellular carcinoma	0.078	0.297	0.077 - 1.147
Pre-LT alcohol history	0.150	2.696	0.699 - 10.399
BMI ≥ 25 kg/m^2 at Bx.	**0.037**	**3.481**	**1.080 - 11.218**

Abbreviation: LT, Liver transplantation; BMI, Body mass index; Bx., Biopsy

국문 초록

서론: 간이식 후 후기 프로토콜 조직검사의 결과에 대해서는 잘 알려져 있지않고, 이식편의 섬유화 및 지방변화는 더욱 그러하다. 본 연구는 간이식 후 후기 프로토콜 조직검사에 기초하여 이식편의 섬유화 및 지방변화를 알아보고, 이들과 관련있는 임상 자료를 파악하고자 하였다.

방법: 2010 년 8 월부터 2012 년 8 월까지 서울대학교병원에서, 간이식 후 1 년 이후에 시행된 프로토콜 조직검사를 받은 간이식 환자를 대상으로 후향적으로 분석하였다. 초음파유도간생검을 시행 후, hematoxylin and eosin 염색과 Masson trichrome 염색을 시행하였다. 이식편의 섬유화 정도는 METAVIR system 으로, 지방변화는 NASH Clinical Resarch Network 의 Pathology Committee 에서 고안한 steatosis scoring system 으로 분류하였고. 이러한 결과를 토대로 이식 전후의 여러 가지 임상자료와의 관계를 분석하였다.

결과: 총 131명의 성인 환자, 174개의 슬라이드가 연구대상이 되었다. 이식편의 섬유화는 23.6% (n = 41), 이들 중 중등도 이상의 섬유화가 46.3%였다(n = 19/41). 다변수분석 결과, 당뇨치료에 관계없이 조직검사 이전 6개월동안의 평균 공복 시 고혈당(≥ 126 mg/dL) (risk ratio 7.260, p = 0.001), CK-19의 ductular reaction 양성이 이식편 섬유화에 따른 두 군간에 유의한 차이를 보였다(risk ratio 7.931, p < 0.001). 중등도 이상의 섬유화의 경우는, CK-19에서 ductular reaction

여부(risk ratio 20.335, $p < 0.001$)와 담도 손상 여부(risk ratio 17.351, $p = 0.001$)가 통계적으로 유의하였다.

이식편의 지방변화는 25.3%였고(n = 44/174), 이들 중 중등도 이상의 지방간은 31.8%였다(n = 14/44). 다변수분석 결과, 남성(risk ratio 3.448, $p = 0.037$), 이식 전 음주(risk ratio 3.543, $p = 0.014$), 조직검사 당시의 비만(BMI ≥ 25 kg/m^2) (risk ratio 2.979, $p = 0.003$)이 통계적으로 유의한 결과를 보였고, 조직검사 당시의 비만은 중등도 이상의 지방변화에서도 의미 있는 관계를 보였다(risk ratio 3.481, $p = 0.037$).

결론: 이식 후 후기 프로토콜 조직검사 상, 이식편의 섬유화 및 지방변화는 상당부분을 차지하는 것으로 나타났다. 본 연구에서는, 당뇨 치료에 관계없이 조직검사 이전 6개월동안 평균 공복 시 고혈당(≥ 126 mg/dL)과 CK-19에서 ductular reaction이 이식편의 섬유화와 유의한 관계를 보였으며, 남성, 이식 전 음주 및 조직검사 당시의 비만이 이식편의 지방변화와 유의한 관계를 보였다. 이러한 결과를 이식환자의 치료과정에 적절히 반영할 수 있겠고, 이는 향후 이식환자의 장기적인 치료성적에 긍정적인 영향을 미칠 수 있을 것이다.

주요어: 간 이식, 프로토콜 간조직검사, 이식편의 섬유화, 이식편의 지방변화, 이식 후 지방간

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