

## Value of Histopathologic Findings of Post-reperfusion Liver Needle Biopsies

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### ABSTRACT

**Background:** Histopathologic changes of post-reperfusion liver needle biopsies in patients with liver transplantation have rarely been reported and most of the previous reports have been in less than 200 cases.

**Objective:** In this study, we evaluated 408 post-perfusion liver needle biopsies for the histopathologic changes attributable to reperfusion injury and compared them with early post-liver transplantation outcome, to find out the value of these findings.

**Methods:** In 408 patients who underwent liver transplantation, post-perfusion liver needle biopsy was taken within one hour of vascular anastomosis. The specimens were fixed in formalin and evaluated by a hepatopathologist blinded to the outcome of transplantation for hepatocellular necrosis, apoptosis, ballooning degeneration, cholestasis, neutrophilic infiltration, and steatosis. These were compared with cold and warm ischemic time, levels of AST, ALT, alkaline phosphatase, bilirubin, presence or absence of rejection, and duration of hospital stay.

**Results:** Hepatocellular ballooning degeneration, apoptosis, and necrosis did not show any significant correlations with early post-transplantation outcome and reperfusion injury. However, presence of neutrophilic infiltration in the post-reperfusion liver biopsy was well correlated with liver function tests and other clinical and paraclinical findings. Presence of steatosis in post-reperfusion liver needle biopsy was also associated with high liver function tests and long hospital stay.

**Conclusion:** Presence of PMN leukocytes in the post-perfusion liver needle biopsy of transplanted liver is associated with poor early outcome and reperfusion injury, so it should be recorded in the pathology report and should be considered a high-risk sign for the clinicians.

**KEYWORDS:** Reperfusion; Biopsy, needle; Liver transplantation; Apoptosis; Necrosis; Histopathologic changes

### INTRODUCTION

Orthotopic liver transplantation is the only choice for treatment of end-stage liver diseases. However, it is a major surgery with many complications, especially when marginal livers with prolonged ischemic time and steatosis are used [1]. One of the main contributing factors in primary allograft failure and poor initial function of transplant-

ed livers is preservation injury after reperfusion of the donor organ, secondary to release of reactive oxygen species and pro-inflammatory mediators [2].

There have been several reported methods for the assessment of the severity and presence of preservation and reperfusion injuries, such as measuring the level of interleukins and cytokines as pro-inflammatory molecules [3]. One of these methods is histopathologic findings in the post-perfusion liver needle biopsies, which have rarely been investigated in the previous studies [4]. There have been many reported

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**Table 1:** Frequency of histopathologic findings in 408 post-perfusion time zero biopsies

Finding	Grade 0	Grade I	Grade II	Grade III
Neutrophilic infiltration	167 (40.9%)	45 (11.0%)	46 (11.2%)	150 (36.8%)
Steatosis	254 (62.2%)	136 (33.3%)	17 (4.2%)	1 (0.0%)
Hepatocellular apoptosis	304 (74.5%)	51 (12.5%)	23 (5.6%)	30 (7.4%)
Hepatocellular necrosis	341 (83.5%)	27 (6.6%)	25 (6.1%)	15 (3.7%)
Hepatocyte ballooning degeneration	221 (54.1%)	166 (40.7%)	17 (4.2%)	4 (1.0%)
Cholestasis	388 (95.1%)	10 (2.4%)	10 (2.4%)	0 (0.0%)

findings in favor of reperfusion injury. However, there is no well documented scoring system. Presence of hepatocellular necrosis, hepatocyte ballooning degeneration, steatosis, cholestasis, lobular neutrophilic infiltration, and hepatocellular apoptosis have been reported as main histopathologic findings in favor of reperfusion injury in post-perfusion (time zero) liver needle biopsies [2].

In this study, we tried to evaluate the histopathologic findings of post-perfusion, time zero liver needle biopsies in 408 transplanted livers.

## PATIENTS AND METHODS

From 2013 to 2015, 408 patients underwent liver transplantation with post-perfusion and time zero liver needle biopsy. Patients with hepatic artery thrombosis, biliary complications, sepsis, fungal infections, and other well-defined causes of transplant injury were excluded from the study. The patients included 268 (65.6%) males and 140 (34.3%) females. The mean±SD age of donors and recipients was 31.1±15 (range: 2–69) and 35.9±17.2 (range: 2–69) years, respectively. These biopsies have been taken within the first hour of vascular anastomosis. All of the specimens were fixed in 10% buffered formalin and embedded in paraffin, then stained by hematoxylin and eosin.

All the liver needle biopsies were examined by a hepatopathologist blinded to the outcome of the liver transplantation for the histopathologic changes of reperfusion injury, i.e., presence and the severity of steatosis, hepatocellular necrosis, neutrophilic infiltra-

tion, apoptosis, ballooning degeneration, and cholestasis as follows [5]: For grading of macrovesicular steatosis, we assumed grade 0 (no to <5%), grade I (5% to <30%), grade II (30% to <60%), and grade III (≥60%). For hepatocellular necrosis, neutrophilic infiltration, and apoptosis, the grading system used was: absent, grade I (1 focus/HPF), grade II (2–3 foci/HPF), and grade III (>3 foci/HPF). And for grading hepatocyte ballooning degeneration and cholestasis, we used the following grading system: absent, grade I (mild), grade II (moderate), and grade III (severe).

All the information about the donor and recipient relating to the outcome of early liver transplantation including cold and warm ischemic times (CIT and WIT), duration of post-liver transplantation hospital stay, presence of rejection, and the last liver function test before discharge were obtained from the patients' clinical charts.

Findings of the post-perfusion liver needle biopsies were compared with the above-mentioned clinical and paraclinical findings to find out the value of main histopathologic findings of post-perfusion time zero liver needle biopsy in predicting the early outcome of the liver transplants.

## RESULTS

We studied 408 patients who underwent liver transplants with post perfusion, time zero liver needle biopsies. Table 1 shows the frequency of histologic findings in these 408 post-perfusion biopsies. Different characteristics that can be indicative of early post-liver transplantation outcome were compared with

**Table 2:** The association between different grades of histologic findings in post-perfusion liver needle biopsies with different characteristics of liver transplant outcome in 408 studied patients

Finding	Grade	CIT* (min-ute)	WIT† (min-ute)	Presence of Rejection	Hospital stay (day)	AST (IU/L)	ALT (IU/L)	Alk (IU/L)	BiI>Total (mg/dL)
PMN infiltrate	0	256±74.4	27±4.7	2	10.6±2.3	42.8±28.6	28.6±23.3	47±33.7	0.4±2
	I	438.2±197.3	44.3±8.7	9	11±5.2	69.9±38.9	65.1±29.3	37.8±42	1.4±2.5
	II	374.9±138.3	41±7.6	6	20.6±6.5	158.5±118.7	133.7±120.3	112±66.3	2.5±4
	III	474±166.7	42.8±9.8	51	85±7.7	198.7±130.8	209.1±130.8	166±75.1	5.9±3.1
	p value	0.05	0.05	0.04	0.05	0.05	0.05	0.04	0.05
Steatosis	0	466.1±164.5	42.6±9.1	43	11.3±3.3	56.9±191.5	133.5±153	42.5±27.9	2.1±2.5
	I	454.2±183.3	43.2±9.2	20	13.5±6.1	216±131.9	218±34.9	45.4±35.1	2.7±3.5
	II	459.4±169.2	42.6±7.6	5	33.4±7.2	240.6±20.2	154.7±145.8	154±145	1.6±1.3
	III‡	720	50	—	14	36	78	236	1.3
	p value	0.1	0.8	0.1	0.05	0.05	0.1	0.05	0.1
Hepato-cellular necrosis	0	461.6±169.4	42.8±9.1	55	13.1±8.1	51±82.5	165±47.9	230±32	2.3±2.8
	I	481.7±194.1	43.4±8.4	4	12.7±6.2	48.4±82.3	102.2±73.9	114±85.9	2±2.5
	II	472.2±167.8	44.3±7.6	6	14.1±5.1	110.5±81.5	123.1±234	123±42	3.2±1.9
	III	432.2±186.1	42.1±6.7	2	13.4±4.1	115.3±78.9	134.2±102	114.1±76	2.3±2.9
	p value	0.5	0.5	0.6	0.8	0.6	0.4	0.3	0.5
Balloon-ing de-genera-tion	0	458.9±169.4	42.5±8.5	39	13.3±8.8	144.1±102.2	189.2±70.6	255±133	2.4±3.4
	I	468.5±178.5	43±9.1	27	12.7±7	60.7±145.4	128.5±158.7	265±49.1	2.2±1.9
	II	457.5±136.2	45±13	2	14.6±6.6	38.3±27.2	91.5±69.4	233±44.2	1.5±0.7
	III	480±49	48.7±14	0	10±1.1	29±24	102.4±30.4	234±48.9	1.9±0.5
	p value	0.9	0.3	0.07	0.6	0.8	0.5	0.3	0.4
Apopto-sis	0	458.5±176.7	43±9.3	50	11.3±3.7	127.9±69.1	177.1±130.2	48.7±36.1	2.3±2.9
	I	479.3±140.9	41.9±8.4	12	14.7±8	35.3±17.2	97.5±84.4	34.4±30.9	2.7±3.2
	II	466.4±146.3	43.2±7.6	1	16.1±16	41±33.9	98.2±80.7	39.2±165	1.9±1.7
	III	478.6±173.2	42.1±7.9	5	12.6±6.1	55.7±78.2	42.8±99.8	45.7±133	2.1±2.9
	p value	0.8	0.8	0.06	0.013	0.8	0.6	0.8	0.6

Continued

Table 2: The association between different grades of histologic findings in post-perfusion liver needle biopsies with different characteristics of liver transplant outcome in 408 studied patients

Finding	Grade	CIT* (min-ute)	WIT† (min-ute)	Presence of Rejection	Hospital stay (day)	AST (IU/L)	ALT (IU/L)	Alk (IU/L)	Bil-Total (mg/dL)
	0	466.4±168.8	42.9±9.1	62	13±8.1	110±86.9	163.4±48.4	236.1±49	2.3±2.8
	I	419.3±189.2	43.1±8.1	6	13.7±7.5	71.2±140.1	148.9±32.1	250.9±34	2.5±3.4
Cho- lestasis	II	450±196.2	36.7±7.5	0	9.5±1.6	66.5±95.8	73±47.6	301±24.5	2.3±3.9
	III‡	—	—	—	—	—	—	—	—
	p value	0.3	0.2	0.4	0.4	0.9	0.9	0.8	0.8

\*CIT: Cold ischemic time

†WIT: Warm ischemic time

‡There was only one patient transplanted with more than 60% steatosis in the post-perfusion liver needle biopsy

§There was no patient with severe cholestasis (grade III) in the biopsies examined

the above histologic findings, i.e., the duration of hospital stay, the level of the last (before discharge) ALT, AST, alkaline phosphatase, and bilirubin level. The mean±SD AST, ALT, and alkaline phosphatase were 148.46±140.80 (range: 8–535), 141±125.37 (range: 7–399), and 147±76.37 (range: 114–236) IU/L, respectively. The mean±SD bilirubin level was 6.5±2.3 (range: 0.3–9.5) mg/dL. The mean±SD hospital stay was 13.04±8.04 (range: 10–121) days. The mean±SD cold and warm ischemic times were 462.79±170.7 (range: 15–840) and 42.85±9.04 (range: 25–80) minutes, respectively. Among the 408 studied patients, 117 patients underwent liver biopsy during their hospital stay; 68 (16.6%) of whom had various grades of acute rejection (the remaining patients were within the normal limit). Different grades of histologic characteristics were compared with different grades and levels of the above-mentioned findings such as the level of ALT and AST, etc.

Table 2 shows the comparison between different grades of histologic findings and the above-mentioned characteristics.

## DISCUSSION

Liver transplantation is the main treatment for patients with end-stage liver failure. In spite of the high success rate and survival rate in this procedure, there are still early complications. One of these complications is attributed to reperfusion injury [6]. There are very few studies regarding histopathologic features of reperfusion injury, predicting by post-reperfusion, time zero liver needle biopsy, which is taken within one hour after vascular anastomosis in liver transplantation [6, 7]. One of the strengths of the current study is its sample size: all previous studies have been performed on less than 200 cases; in the current study, we included 408 patients.

We observed significant (p<0.05) correlation between the presence of neutrophils in the liver with CIT, WIT, rejection, duration of hospital stay and also early LFT levels. This finding was also shown in some of the previous

studies as the most important characteristic predicting reperfusion injury [2]. In some reports, presence of hepatocellular necrosis has been introduced as one of the main features of severe reperfusion injury and correlated with early post-liver transplant outcome [8]. However, in our study, the majority of cases (83.5%) failed to show any evidence of hepatocellular necrosis and the correlation between this histopathologic finding and other features was not significant. This was also true for liver steatosis, i.e., more than 80% of our patients showed less than 30% of steatosis with statistically significant correlation with post-liver transplantation AST and alkaline phosphatase level as well as duration of hospital stay ( $p < 0.05$ ). In some reports, steatosis did not show any major effect on the outcome of liver transplantation after seven days; the authors believe that the presence of steatosis in post-reperfusion liver needle biopsy has minor effects on the LFT and other clinical findings in the first seven days [9, 10]. In the studied 408 patients, about 25% of the biopsies showed different grades of hepatocellular apoptosis. However, there was no correlation between these findings and early post-liver transplantation complications. In some of the previous studies, this finding was well correlated with post-liver transplant LFT levels [1]. We also evaluated hepatocyte ballooning degeneration and cholestasis, none of which was correlated with early post-liver transplant findings in favor of reperfusion injury.

In conclusion, the most important and predicting histopathologic finding in post-perfusion liver needle biopsy was infiltration of neutrophils, which should be highly appreciated in pathology report and should be considered an important high-risk sign by the clinicians.

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## REFERENCES

1. Shahbazi N, Haeri H, Nasiri Toosi M, et al. Correlation of Histopathologic Findings of Non-Graft Threatening Preservation/Reperfusion Injury in Time-Zero Liver Needle Biopsies With Short-Term Post-transplantation Laboratory Alterations. *Hepat Mon* 2015;**15**:e30008.
2. Kocbiyik A, Demirhan B, Sevmis S, et al. Role of postreperfusion subcapsular wedge biopsies in predicting initially poor graft function after liver transplantation. *Transplant Proc* 2009;**41**:2747-8.
3. Nikeghbalian S, Mansoorian MR, Hosseini SMV, et al. Reduction of the severity of ischemia reperfusion induced pancreatitis by ischemic preconditioning of the liver. *Saudi J Kidney Dis Transpl* 2009;**20**:1010-5.
4. Gaffey MJ, Boyd JC, Traweek T, Ashraf Ali M, Rezeig M, Caldwell SH, et al. Predictive value of intraoperative biopsies and liver function tests for preservation injury in orthotopic liver transplantation. *Hepatology* 1997;**25**:184-189.
5. Ali JM, Davies SE, Brais RJ, et al. Analysis of ischemia/reperfusion injury in time zero biopsies predicts liver allograft outcomes. *Liver Transpl* 2015;**21**:487-99.
6. Busquets J, Serrano T, Fugueras J, et al. Influence of donor postreperfusion changes on graft evolution after liver transplant. *Transplant Proc* 2002;**34**:252-3.
7. Busquets J, Fugueras J, Serrano T, et al. Postreperfusion biopsy changes predict biliary complications after liver transplantation. *Transplant Proc* 2002;**34**:256-8.
8. Angelescu M, Hofmann W, Zapletal C, et al. Histomorphological analysis of preservation injury as determinant of graft quality in clinical liver transplantation. *Transplant Proc* 1999;**31**:1074-76.
9. Afonso RC, Saad WA, Parra OM, et al. Impact of steatotic grafts on initial function and prognosis after liver transplantation. *Transplant Proc* 2004;**36**:909-11.
10. Xu J, Sayed BA, Casas-Ferreira AM, et al. The Impact of Ischemia/Reperfusion Injury on Liver Allografts from Deceased after Cardiac Death versus Deceased after Brain Death Donors. *PLoS One* 2016;**11**:e0148815.