Прогностическая роль уровней внеклеточных гистонов H3 для диагностики ранней дисфункции трансплантата после трансплантации печени

Н. Г. Новрузов

Центральный таможенный госпиталь, г. Баку, Азербайджанская Республика

Prognostic role of levels of extracellular histones H3 for diagnosis of early dysfunction of transplant after hepatic transplantation

N. G. Novruzov

Central Customs Hospital, Baku, Republic of Azerbaijan

Реферат

Цель. Целью настоящего исследования явилось определение прогностической роли уровней внеклеточных гистонов для диагностики ранней дисфункции трансплантата после трансплантации печени.

Материалы и методы. В исследование были включены результаты лечения 93 реципиентов, после трансплантации печени из живых доноров. Образцы крови пациентов собирали на 1-й день после операции, и уровни гистонов в плазме измеряли с помощью наборов для гистонов H3 сэндвич-ELISA анализа. У 19 (20,4%) пациентов, ранняя дисфункция трансплантата была диагностирована на 7-й день после операции в соответствии с критериями Ol-thoff, основанными на функциональных тестах печени и профилях коагуляции. У остальных 74 (79,6%) реципиентов не наблюдали ранней дисфункции трансплантата.

Результаты. Уровни циркулирующих гистонов были ниже у пациентов с ранней дисфункцией трансплантата $0,808 \pm 0,026$, чем у реципиентов без дисфункции $0,820 \pm 0,017$ ($p = 0,727$). Уровни общего гистона H3 после 24 часов операции не имели строгого прогностического значения с AUC 0,477 для дисфункции печени. Значение AUC общего билирубина (AUC 0,685) при прогнозировании ранней дисфункции трансплантата превосходило другие функциональные тесты печени, и было меньше, чем СРБ (AUC 0,705). Оптимальное пороговое значение общего билирубина, полученное из анализа кривых ROC, составило 4,5 и превзошло все другие параметры с чувствительностью 94,4% для прогнозирования ранней дисфункции печени. Послеоперационный уровень нейтрофилов и СРБ были определены как независимые факторы риска ранней дисфункции трансплантата. Нейтрофилы имели более высокую прогностическую ценность для дисфункции печени, чем другие параметры в течение 24 часов после операции ($p = 0,016$).

Выводы. В совокупности уровни внеклеточного гистона H3 были снижены, уровни общего билирубина и СРБ были повышены у пациентов с ранней дисфункцией трансплантата, что эти данные можно использовать в качестве ранних предикторов повреждения ткани печени и ранней дисфункции аллотрансплантата у пациентов после трансплантации печени.

Ключевые слова: трансплантация печени; дисфункция трансплантата; гистоны.

Abstract

Objective. The aim of this study was to determine the prognostic role of extracellular histones in the diagnosis of early graft dysfunction after liver transplantation.

Materials and methods. The 93 recipients undergoing LDLT were enrolled in this prospective study. Blood samples of patients were collected on postoperative day 1 and histone levels in the plasma samples were measured with Total Histone H3 sandwich ELISA kits. 19 (20.4%) subjects had early graft dysfunction (EAD) which was diagnosed on postoperative day 7 according to Ol-thoff's criteria, based on liver function tests and coagulation profiles. Other 74 (79.6%) recipients did not have EGD.

Results. Levels of circulating histones were depressed in patients with EGD ($0,808 \pm 0,026$, 95% (CI) 0.752–0.864) than in patients without EGD ($0,820 \pm 0,017$, 95% (CI) 0.786–0.854) ($p = 0.727$). These differences were not significant. The sources of histones in the circulation are not etiologies specific and levels of total histone H 3 after 24 h of operation had not stronger predictive value with AUC 0.477 (95 % CI 0.329 to 0.625) for liver dysfunction.

The AUC value of the total bilirubin (AUC 0.685, 95 % CI 0.546 to 0.825) in predicting early graft dysfunction outperformed other LFTs and was less than CRP (AUC 0.705, CI 0.573 to 0.838).

The optimal cutoff value of total bilirubin obtained from the analysis of ROC curves was 4.5 and surpassed all other parameters with a sensitivity of 94.4% and a specificity of 40.7% respectively for prognoses of EGD ($P = 0.012$).

The univariate analysis determined that postoperative neutrophils level and CRP were identified as independent risk factors for early graft dysfunction. Neutrophils had a higher predictive value for liver dysfunction than any other parameter within 24 h (Odds ratio (OR) 16.3; 95% CI: 1.7–156.3, $p = 0.016$).

Conclusion. Collectively, extracellular histone H3 levels were depressed, total bilirubin and CRP levels were elevated in patients with EGD, which can be used as early predictors for liver tissue damage and early allograft dysfunction in patients after liver transplantation.

Keywords: Liver transplantation; graft dysfunction; histones

Introduction

Liver transplantation is a life-saving treatment for patients with end stage liver disease. Initial dysfunction of the graft in the immediate postoperative period influences patient condition.

The definition of early allograft dysfunction (EAD) based on the previously published studies, and is thought to be reflective of liver functional status at postoperative period. Early allograft dysfunction is a clinical criterion of an important endpoint that tries to determine specific donor or recipient risk factors after liver transplantation [1–5].

Olthoff et al. performed a cohort study of deceased donor liver transplants, examined and updated definition of EAD, and correlated this definition with graft and patient outcome. EAD was recognized as the presence of one or more of the postoperative laboratory analyses reflective of liver function: elevated bilirubin levels $\geq 10\text{mg/dL}$ on day 7, international normalized ratio >1.6 on day 7, and elevated serum alanine aminotransferase (ALT) or aspartate aminotransferases (AST) $>2000\text{ IU/L}$ within the first 7 days [1].

Several clinical randomized controlled trials now use liver graft dysfunction or evolution of transaminases as a primary outcome for patients after liver transplantation. Total bilirubin levels and INR on early postoperative days still reflect the status of the recipient at the time of transplantation, but aminotransferases on postoperative days 1–7 are more reflect the function of the graft [3].

The MEAF score provides additional information on prognosis about expected posttransplant outcomes. The MEAF score reflect biochemical results obtained in the first 3 days after operation, and many more factors that will influence transplant function in the later postoperative period [6].

Under conditions of cellular stress and ischemic injury, histones can be released from damaged cells and activated immune cells such as neutrophils into the blood. The pathological role for extracellular histones in various disease processes has been investigated [2, 7].

The literature has shown that cell free histones may be related to the functional status of liver. However, little is known about clinical relevance of extracellular histones with early graft dysfunction after liver transplantation [7, 8].

In this study, we aimed to identify extracellular histones H3 analysis may provide clinicians information on the function and cellular state of a graft and may predict the event of EAD on postoperative day 1 after LDLT.

Materials and methods

After exclusion of cancer and pediatric patients and patients with acute liver failure status, 93 recipients undergoing LDLT at the Liver Transplantation Institute of Malatya, Turkey between November 2018 and October 2019 were enrolled to this prospective study.

The grafts were obtained from living donors. Main reasons for transplantation were cryptogenic cirrhosis ($n=30$, 32%), HBV ($n=27$, 29%), Autoimmune cirrhosis ($n=9$, 9.6%), Alcoholic cirrhosis ($n=8$, 8.6%), Metabolic cirrhosis ($n=7$, 7.5%) and others ($n=12$, 12.9%).

Within 7 postoperative days, the primary allograft function was assessed based on Olthoff's criteria. Grafts meeting one or more of this criteria was considered as Early Graft Dysfunction. 93 patients received LDLT and 19 (20.4%) subjects had EGD which diagnosed on postoperative day 7 based on liver function tests and coagulation profiles, as previously mentioned, the other 74 (79.6%) recipients did not have early graft dysfunction.

Elevated bilirubin and INR were the most frequent variables noted in recipients meeting our definition and 13 (68.4%) of recipients with EAD met elevated bilirubin and INR together.

The primary outcome was the association between histones expression in recipients' samples and subsequent development of early organ dysfunction in the immediate period following transplantation within first 7 days after transplantation. The LFTs assessed as secondary outcomes.

Blood samples. The blood samples were collected and centrifuged immediately on postoperative day 1, to obtain plasma following the kit instruction and stored at -80°C until further Histones H3 analyses.

Total Histone H3 levels in the plasma samples of the patients were measured with PathScan Total Histone H3 sandwich ELISA kit (Cell Signaling Technology, US) according to manufacturer's instructions.

Postoperative laboratory variables included the conventional liver function tests (LFTs), serum Albumin, international normalized ratio (INR), lactate dehydrogenase (LDH), CRP, PCT, NLR and Ammonium, were measured on 1, 3, 5 and 7 postoperative days using routine biochemical methods.

We investigate to assay extracellular histones activity and indicators of systemic inflammation such as WBC, neutrophil-lymphocyte ratio (NLR), CRP, PCT levels, which reflect activation of immune cells particularly neutrophils, during in the postoperative period.

Statistical analysis. The continuous variables data were presented as the mean $\pm$ standard deviation (SD), and were compared by Mann-Whitney U test (2 groups). The categorical data were presented as frequencies and compared using the chi-square test or the Fisher's and Snedecor's exact test. Kruskal-Wallis test and Spearman rank correlation were used. To identify an independent predictor of postoperative EAD, linear logistic regression analysis was performed. A receiver operator characteristic curve (ROC) was constructed to compare the predictive accuracy of the identified variables. Optimal cut-off values for sensitivity and specificity for each parameter were derived from the ROC curves. A p -value <0.05 was considered to be significantly different. The analyses were performed using SPSS® version 22.0 (IBM, Armonk, New York, USA).

Results

We analyzed both donor and recipient factors to eliminate parameters related to graft dysfunction. Donor variables: age, gender, BMI, graft type, liver functional tests, inflammatory markers and donor histones H3 levels were analyzed to identify risk factors for EGD in recipients.

Table 1. Univariate Association of Risk Factors with EGD

Parameters	EAD (n=19)	Non EAD (n=74)	P-value
Donor age	29.4±1.5	29.3±1.0	0.947
Donor height, sm	172.4±2.4	172.2±0.9	0.921
Donor weight, kg	72.5±2.5	72.2±1.4	0.914
Donor BMI	24.5±0.4	24.3±0.3	0.879
Donor Total Histones H3 µg/ml	0.822±0.034	0.831±0.021	0.831
Recipient variables			
Age	43.6 ± 2.8	51.0 ± 1.4	0.013
Gender F/M	6/13	26/48	0.712
BMI	25.4 ± 1.4	26.1 ± 0.5	0.536
MELD	19.6 ± 1.8	18.2 ± 0.6	0.348
MEAF	6.49 ± 0.39	5.65 ± 0.18	0.036
CTP	9.26 ± 0.36	8.9 ± 0.19	0.375
Child B, %	52.6	72.6	
Child C, %	47.4	27.4	
Graft size, mg	747.5 ± 55.6	795.1 ± 20.5	0.325
GRWR, %	1.06 ± 0.07	1.08 ± 0.03	0.766

Table 2. Baseline laboratory characteristics and clinical outcomes of recipients

Factors	Postoperative day 1			Postoperative day 7		
	EGD	non EGD	P	EGD	Non EGD	P
Ammonium	165.6 ± 21.9	128.9 ± 5.7	0.023	101±9.8	74±2.3	0.000
Bilirubin Total	8.82 ± 1.19	6.14±0.5	0.019	10.15±1.25	3.46±0.30	0.000
AST	500.9±133.3	319.5±21.2	0.030	176.2±44.6	57.6±7.0	0.000
ALT	528.2±119.9	371.5±26.0	0.055	351.8±79.2	134.3±11.4	0.000
Kreatinin	87.5 ± 7.2	110.6 ± 5.7	0.040	98.0±14.2	78.3±2.6	0.035
BUN	19.9 ± 2.5	25.7 ± 1.2	0.027	24.4±4.2	32.1±2.3	0.11
Na	139.7 ± 0.9	136.0 ± 0.5	0.001	137.7±1.0	135.1±0.6	0.028
WBC	13.9±1.8	11.7±0.6	0.158	16.9±2.4	10.1±0.7	0.000
PLT	151.3 ± 28.2	88.7 ± 6.2	0.002	99.1±0.95	100.1±8.4	0.951
Neutrophils	15.32 ± 3.9	10.06 ± 5.7	0.028	14.24±2.2	7.63±0.6	0.000
NLR	10.9±1.4	13.3±0.9	0.198	12.1±2.4	8.8±0.9	0.123
CRP	19.5±5.2	11.0±2.7	0.008	51.5±10.4	38.9±5.2	0.251
INR	2.95 ± 0.32	2.40 ± 0.11	0.040	1.82±0.1	1.18±0.02	0.000
APTT	57.6 ± 9.2	44.1 ± 2.6	0.055	45.8±6.7	26.4±0.7	0.000
LDH	561.1±119.5	397.6±32.9	0.068	437.1±48.4	252.8±11.8	0.000

The relationship of donor and recipient variables with EGD is graphically presented in *Table 1*. A univariate analysis revealed no donor related variables, which can be risk factors for postoperative early liver dysfunction in recipients.

Of the recipient parameters, no statistical significance was observed for patient body mass index (BMI), gender, graft size and graft to recipient weight ratio (GRWR) between groups except for the recipient age, which were statistically higher in the non EAD group (51.0 ± 1.4 versus 43.6 ± 2.8, P=0.013).

The MELD and MEAF scores appeared to be risk factors for liver dysfunction after transplantation. Although MELD score was higher in EGD group than non EGD group, in univariate analysis this differences was not statistically significance, 19.6 ± 1.8 and 18.2 ± 0.6 respectively (P = 0.348).

Although the patients with Child C were higher in the EAD group than other, the CTP score did not differ significantly be-

tween groups (47.4% versus 27.4%, P=0.39). The MEAF score was significantly higher in EGD group patients than non EGD group, respectively 6.49 ± 0.39 and 5.65 ± 0.18 (P = 0.036).

Totally nine patients had a graft recipient weight ratio (GRWR) less than 0.8%, and the mean dates for GRWR between groups were not differ significantly 1.06 ± 0.07 and 1.08 ± 0.03 respectively (P = 0.766).

Among the 93 recipients, the mortality within 7 postoperative days were developed in EAD group and was observed in 1 (5.26%) patient, in non-EAD groups was observed in 2 (2.7%) cases.

The clinical characteristics of patients with EGD versus patients without EGD were compared. Baseline laboratory characteristics and clinical outcomes for each group are illustrated in *Table 2*.

On postoperative day 1, EAD group showed significantly worse coagulation profiles than non-EAD group. INR value

a: Comparison of circulating histone levels between groups, $P > 0.050$ (Mann–Whitney U test).
b: Comparison of circulating histone levels between different etiologies ($P > 0.050$).

was 2.95 ± 0.32 and 2.40 ± 0.11 ($P=0.040$) and APTT was 57.6 ± 9.2 and 44.1 ± 2.6 ($P = 0.055$) respectively. These differences at one week were significant ($P=0.000$).

Similarly EAD group demonstrated significantly poorer liver function tests on postoperative day 1. Patients with EGD had significantly different higher blood levels of total bilirubin and transaminases (Table 2).

The ALT level reached its mean peak of 528.2 ± 119.9 U/l the first day after the operation in patients with EGD, and 371.5 ± 26.0 , in patients without EGD ($P=0.055$), and decreased to 351.8 ± 79.2 U/l versus 134.3 ± 11.4 U/l, respectively on day 7 after the operation ($P=0.000$).

The postoperative mean peak serum AST levels in both groups were observed on the first day in the adequate and delayed liver recovery groups 500.9 ± 133.3 U/l and 319.5 ± 21.2 U/l respectively ($P=0.030$). The AST levels decreased to 176.2 ± 44.6 versus 57.6 ± 7.0 ($P=0.000$) 7 days later. ALT and

AST levels significantly differ between the groups (Table 2).

The mean peak serum total bilirubin level on a postoperative day 1 were (8.82 ± 1.19 versus 6.14 ± 0.5 mg/dl) ($P=0.019$) and significantly increased on a postoperative day 7 in the EGD group when compared with the non EGD group (10.15 ± 1.25 versus 3.46 ± 0.30 mg/dl) ($P=0.000$).

The liver enzyme elevation and Hyperbilirubinemia in the immediate postoperative period declined smoothly over a week in patients without EGD.

The mean serum Ammonium level reached its median peak on day 3 after the operation and was higher in the EGD group over time ($P=0.023$), normalized within one week in patients without EGD but differences were significant ($P=0.000$).

In addition, we evaluated the degree of systemic inflammation in patients after liver transplantation by measuring the WBL, Neutrophils, NLR, CRP and PCT. We found that the WBL and Neutrophils markedly increased in EGD recipients

Table 3. Total histones H 3 levels between different subgroups

Recipient variables	EAD (n=19) Total histones H3, µg/ml	Non EAD (n=74) Total histones H3, µg/ml	P-value
MELD<18	0.79 ± 0.03 (n=8)	0.82 ± 0.02 (n=30)	0.635
MELD≥18	0.83 ± 0.05 (n=11)	0.82 ± 0.03 (n=32)	0.483
Child B	0.79 ± 0.03 (n=10)	0.83 ± 0.02 (n=45)	0.247
Child C	0.83 ± 0.04 (n=9)	0.80 ± 0.05 (n=17)	0.396
GRWR ≥1	0.81 ± 0.05 (n=9)	0.83 ± 0.02 (n=41)	0.747
GRWR<1	0.80 ± 0.03 (n=10)	0.81 ± 0.03 (n=21)	0.917
BMI normal	0.82 ± 0.03 (n=12)	0.81 ± 0.02 (n=25)	0.962
BMI > normal	0.80 ± 0.06 (n=7)	0.83 ± 0.03 (n=37)	0.593

at 24 hour after liver transplantation as compared with their levels in non EGD group. These differences were significant on day 7 (Table 2).

Levels of circulating histones in the both group patients are shown in Figure 1a. Postoperative extracellular Histones H3 levels were depressed in patients with EGD (mean 0.808 ± 0.026 , 95% (CI) 0.752–0.864) than in patients without EGD (mean 0.820 ± 0.017 , 95% (CI) 0.786–0.854) ($P=0.727$). These differences were not significant.

Levels of Total histone H3 after 24 h of operation had not stronger predictive value with AUC 0.477, 95% CI 0.329 to 0.625 ($P=0.763$). In addition, we compared extracellular histone levels among recipients with different etiologies and found no significant difference (all $P>0.05$), thus suggesting that the sources of histones in the circulation are not etiologies specific (see Figure 1b).

In Table 3 the overall Histone H 3 level were calculated separately for patients with low (<18) or high (≥ 18) MELD score, for Child B or Child C patients, for with low (<1) or high (≥ 1) GRWR and for recipients with normal and high BMI index.

There was no significant difference between histone levels in Child B and Child C patients who underwent LT, 0.81 ± 0.03 $\mu\text{g/ml}$ versus 0.82 ± 0.04 $\mu\text{g/ml}$ respectively, $P>0.05$). The same time extracellular histone levels were not significant differ in patients with different graft size, when recipients with $\text{CRWR}<1$ compared with the patients having $\text{CRWR} \geq 1$ (0.82 ± 0.03 $\mu\text{g/ml}$ versus 0.80 ± 0.02 $\mu\text{g/ml}$, $P>0.05$). The differences for all these parameters were not significantly and the results show the prognostic usefulness of serum histone H3 levels; independently of graft status.

The AUC values for potential predictors of EGD are summarized in Table 4. The values for all the biomarkers were moder-

Table 4. AUC values for potential predictors of early graft dysfunction (area under the curve)

Variables on day 1	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				lower bound	upper bound
CRP	0.705	0.068	0.009*	0.573	0.838
Ammonium	0.586	0.080	0.260	0.428	0.740
Total histones H3	0.477	0.076	0.763	0.329	0.625
BUN	0.262	0.069	0.002*	0.126	0.398
Kreatinin	0.344	0.064	0.041*	0.218	0.470
LDH	0.663	0.074	0.032*	0.518	0.808
Bilirubin Total	0.685	0.071	0.015*	0.546	0.825
Direct bilirubin	0.683	0.072	0.016*	0.541	0.824
Na	0.747	0.061	0.001*	0.627	0.867
Er	0.751	0.069	0.001*	0.615	0.887
Hb	0.660	0.074	0.036*	0.515	0.804
Ht	0.728	0.075	0.003*	0.581	0.874
PLT	0.622	0.084	0.110	0.456	0.787
INR	0.589	0.089	0.242	0.415	0.764
Albumin	0.375	0.071	0.101	0.236	0.515

Table 5. Factors sensitivity and specificity for prediction of early graft dysfunction

Factors	Cut off point ROC curves	Sensitivity $\pm$ mp	Specificity $\pm$ mp	Chi-Square P-value
Ammonium	> 190.5	31.6 ± 10.7	90.3 ± 3.8	0.019*
CRP	> 4.5	94.4 ± 5.4	40.7 ± 6.4	0.012*
BUN	< 20.5	78.9 ± 9.4	72.6 ± 5.7	0.000***
Kreatinin	< 93.5	89.5 ± 7.0	54.8 ± 6.3	0.002*
LDH	> 452	52.6 ± 11.5	80.6 ± 5.9	0.004**
Total Bilirubin	> 8.06	47.4 ± 11.5	87.1 ± 4.3	0.001**
AST	> 367.5	52.6 ± 11.5	69.4 ± 5.9	0.080
ALT	> 580.5	36.8 ± 11.1	88.7 ± 4.0	0.010**
Na	> 135.5	84.2 ± 8.4	58.1 ± 6.3	0.003**
Hemoglobin	> 115	42.1 ± 11.3	87.1 ± 4.3	0.005**
Hematocrit	> 32.5	68.4 ± 10.7	82.3 ± 4.9	0.000**
Neutrophils	> 20.5	21.1 ± 9.4	98.4 ± 1.6	0.011*
PLT	> 115	52.6 ± 11.5	82.3 ± 4.9	0.002**
INR	> 2.65	57.9 ± 11.3	80.6 ± 5.0	0.001*
APTT	> 64.5	31.6 ± 10.7	91.9 ± 3.5	0.009**

ate (range 0.262–0.751) in predicting early graft dysfunction.

Levels of Total bilirubin AUC after 24 h of operation outperformed other LFTs and had stronger predictive value (AUC 0.685, 95% CI 0.546 to 0.825) than either BUN (AUC 0.262, CI 0.126 to 0.398) or Creatinine (AUC 0.344, CI 0.218 to 0.470), but was comparable to LDH (AUC 0.663, CI 0.518 to 0.808), and was less than CRP (AUC 0.705, CI 0.573 to 0.838).

The cutoff point value obtained from the analysis of ROC curves for the variables with sensitivity and specificity for examining the possible relationship between early graft dysfunction were described in *Table 5*.

The calculated cut-off value for CRP more than 4.5 with the sensitivity of 94.4% and specificity of 40.7% ($P=0.012$) outperformed other LFTs. Total bilirubin more than 8.06 mg/dl with the sensitivity of 47.4% and specificity of 87.1% ($P=0.001$), the cut-off value for Ammonium more than 190.5 $\mu\text{g/dl}$ with the sensitivity of 31.6 % and specificity of 90.3 % ($P=0.019$) and for the PLT level at 24 h with a cut-off of $115.0 \cdot 10^3/\text{l}$ the sensitivity, and specificity with 52.6% and 82.3% respectively ($P=0.130$) were significant by Chi-Square test (*Table 5*).

The univariate analysis determined that postoperative neutrophils level and CRP identified as an independent risk factor for early graft dysfunction. Neutrophils had a higher predictive value for liver dysfunction than any other parameter within 24 h (Odds ratio (OR) 16.3; 95% CI: 1.7–156.3, $P=0.016$). Predictive value of the neutrophils was stronger than CRP (OR) 11.7; 95% CI: 1.5–93.6, $P=0.021$), Total bilirubin (OR) 6.1; 95% CI: 1.9–19.5, $P=0.002$), Ammonium (OR) 4.3; 95% CI: 1.2–15.5 $P=0.026$), and INR (OR) 5.7; 95% CI: 1.9–17.3, $P=0.002$).

Both INR and PLT within 24 h had reasonable predictive values, but sensitivity was much lower than CRP.

Discussion

A simple definition of EGD using objective posttransplant criteria was highly associated with graft loss and patient mortality and can be used as an endpoint in translational studies aiming to identify patients with clinical expression of suboptimal liver function [1–4].

Overall incidence of EGD ranging from 5.2% to 36.3% with an EAD associated mortality reaching 18.8%. In our study the incidence of EAD was 20.4%.

Some authors defined donor age and MELD score as significant risk factors for early allograft dysfunction. The finding of an association between increasing risk of development of EAD and increasing MELD demonstrates that it is not just donor factors play a role in graft dysfunction following transplantation [1, 3, 6, 8, 9].

The etiology of EGD is multifactorial. Donor related factors such as age, BMI, and transplant-related factors such as recipient age, MEAF, MELD scores, GRWR, ischemia/reperfusion (I/R) injury have been proposed as common risk factors for EGD which can lead to the graft dysfunction [10–13].

In the current study a univariate analysis revealed no donor related variables, which can be risk factors for postoperative early liver dysfunction in recipients (*Table 2*), which can be explained due to our donor evaluation program [14].

The MEAF score has been proposed as a measure of severity of early graft dysfunction after liver transplantation. Pareja et al, who designed the MEAF score, recommended high MEAF scores could signify a risk for the development of primary non-function [6]. In our study the MEAF score was significantly higher in EGD group patients than non EGD group (*Table 1*).

Many studies have confirmed that I/R injury of the liver, lungs and kidney caused an elevation of extracellular histones in the circulation [15–17].

Xiuhui Li et.al. observed that patients with PGD had significantly higher levels of extracellular histones than patients without PGD after transplantation, thereby suggesting that extracellular histones are an important cause for PGD [8].

In the present study, we have evaluated the extracellular histones H3 between EAD and non-EAD recipients as early as postoperative day 1, hypothesized that the quantitative measurement of extracellular histone concentration may provide clinicians information on the function and cellular state of a graft.

In the current study we evaluated a range of extracellular histone H3 levels, and clinically-used LFTs and inflammation markers and indices in relation to early graft dysfunction. We have evaluated the extracellular Histones H3 levels between different MELD scores, BMI index, different graft to recipient weight ratio, Child B and Child C patients among EGD and non-EGD recipients at early postoperative day 1.

Despite histone H3 levels were depressed at first day after operation in patients with EGD, than that in the patients without EGD, it was not significant on univariate analysis (0.820 ± 0.017 , 95% (CI) 0.786–0.854) ($P=0.727$). In our study, histones H3 alone, were found not effectively differentiate EAD from non-EAD in recipients on postoperative day 1.

In order to try to explain these results; the clinical laboratory parameters of patients with EGD were compared with those of patients without EGD. Amongst the clinical laboratory parameters; Total bilirubin; ALT, AST, LDH, Ammonium, INR, CRP and NLR; levels were significantly higher in patients with EGD; compared with non EGD group. Thus; indices of inflammation and liver damage were associated with patients in EGD group.

In our study, a Neutrophils, Ammonium, Total bilirubin and LDH more specifically, were found to effectively differentiate EAD from non-EAD on postoperative day 1 (*Table 5*).

Previously it has been reported that levels of extracellular histones are closely associated with immune cell activation and pronounced systemic inflammation [4, 17–19].

In addition, we detected remarkable immune cell activation and a significant inflammatory response after transplantation. We observed that in patients with EGD, CRP and neutrophils levels were both elevated significantly at first day after transplantation, as compared with non EGD group and showed a decreasing trend within a week after transplantation, but were still higher in patients with EGD than without EGD.

The ROC analysis revealed that CRP level and clinically available parameters may serve as an excellent early predic-

tor panel for EAD, which may become applicable to clinical practice. CRP level better than the other clinically available markers, exhibit a high prognostic accuracy in the prediction of EGD within 7 postoperative days with AUCs of 0.705, CI 0.573 to 0.838) and Neutrophils had a higher predictive value for liver dysfunction than any other parameter within 24 h ((OR) 16.3; 95% CI: 1.7–156.3, $P = 0.016$).

Total bilirubin gives an AUC of 0.685 (95 % CI 0.546 to 0.825) in the prediction of EGD and it was less than CRP (AUC 0.705, CI 0.573 to 0.838).

Our results show depression, of circulating H3 levels in recipients after transplantation. However, this discrepancy may be due to the time point of blood collection. We suggest that hepatocyte damages occur 24 h after resection and therefore do not directly affect histone levels within the first 24 h.

Collectively, these findings suggested that the elevated CRP and Total bilirubin in patients on postoperative day 1 is highly predictive of EAD with AUCs of 0.705 and 0.685, better than the other clinically available markers.

Consistent with this, extracellular histone H3 levels were depressed, total bilirubin and CRP levels were elevated in patients with EGD, which can be used as early predictors for liver tissue damage and early allograft dysfunction in patients after living donor liver transplantation.

References

1. Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transpl.* 2010 Aug;16(8):943–9. doi: 10.1002/lt.22091. PMID: 20677285.
2. Lee DD, Croome KP, Shalev JA, Musto KR, Sharma M, Keaveny AP, et al. Early allograft dysfunction after liver transplantation: an intermediate outcome measure for targeted improvements. *Ann Hepatol.* 2016 Jan–Feb;15(1):53–60. doi: 10.5604/16652681.1184212. PMID: 26626641.
3. Jochmans I, Fieuwis S, Monbaliu D, Pirenne J. "Model for Early Allograft Function" Outperforms "Early Allograft Dysfunction" as a Predictor of Transplant Survival. *Transplantation.* 2017 Aug;101(8):e258–e264. doi: 10.1097/TP.0000000000001833. PMID: 28557956.
4. Tsai HI, Lo CJ, Lee CW, Lin JR, Lee WC, Ho HY, et al. A panel of biomarkers in the prediction for early allograft dysfunction and mortality after living donor liver transplantation. *Am J Transl Res.* 2021 Jan 15;13(1):372–82. PMID: 33527031; PMCID: PMC7847515.
5. Pomposelli JJ, Goodrich NP, Emond JC, Humar A, Baker TB, Grant DR, et al. Patterns of Early Allograft Dysfunction in Adult Live Donor Liver Transplantation: The A2ALL Experience. *Transplantation.* 2016 Jul;100(7):1490–9. doi: 10.1097/TP.0000000000001240. PMID: 27326811; PMCID: PMC4943080.
6. Pareja E, Cortes M, Hervás D, Mir J, Valdivieso A, Castell JV, et al. A score model for the continuous grading of early allograft dysfunction severity. *Liver Transpl.* 2015 Jan;21(1):38–46. doi: 10.1002/lt.23990. Epub 2014 Nov 24. PMID: 25204890.
7. Wen Z, Lei Z, Yao L, Jiang P, Gu T, Ren F, Liu Y, Gou C, Li X, Wen T. Circulating histones are major mediators of systemic inflammation and cellular injury in patients with acute liver failure. *Cell Death Dis.* 2016 Sep 29;7(9):e2391. doi: 10.1038/cddis.2016.303. PMID: 27685635; PMCID: PMC5059889.
8. Li X, Gou C, Pang Y, Wang Y, Liu Y, Wen T. Extracellular histones are clinically associated with primary graft dysfunction in human liver Transplantation. *RSC Adv.* 2019;9:10264–71. DOI: 10.1039/C9RA00425D.
9. Deschenes M. Early allograft dysfunction: causes, recognition, and management. *Liver Transpl.* 2013 Nov;19 Suppl 2:S6–8. doi: 10.1002/lt.23746. PMID: 24038766.
10. Kawai C, Kotani H, Miyao M, Ishida T, Jemal L, Abiru H, et al. Circulating Extracellular Histones Are Clinically Relevant Mediators of Multiple Organ Injury. *Am J Pathol.* 2016 Apr;186(4):829–43. doi: 10.1016/j.ajpath.2015.11.025. Epub 2016 Feb 12. PMID: 26878212.
11. Bruns H, Heil J, Schultze D, Al Saeedi M, Schemmer P. Early markers of reperfusion injury after liver transplantation: association with primary dysfunction. *Hepatobiliary Pancreat Dis Int.* 2015 Jun;14(3):246–52. doi: 10.1016/s1499–3872(15)60384–8. PMID: 26063024.
12. Molnar MZ, Joglekar K, Jiang Y, Cholankeril G, Abdul MKM, Kedia S, et al. Association of Pretransplant Renal Function With Liver Graft and Patient Survival After Liver Transplantation in Patients With Non-alcoholic Steatohepatitis. *Liver Transpl.* 2019 Mar;25(3):399–410. doi: 10.1002/lt.25367. PMID: 30369023; PMCID: PMC6709989.
13. Ali JM, Davies SE, Brais RJ, Randle LV, Klinck JR, Allison ME, Chen Y, Pasea L, Harper SF, Pettigrew GJ. Analysis of ischemia/reperfusion injury in time-zero biopsies predicts liver allograft outcomes. *Liver Transpl.* 2015 Apr;21(4):487–99. doi: 10.1002/lt.24072. PMID: 25545865.
14. Novruzov N, Bayramov N, Mammadov E. Preoperative Evaluation of Liver Parenchyma of potential donors in Living Donor Liver Transplantation. *Transplantation Proceedings.* 2019;51(7):2379–82. doi: 10.1016/j.transproceed.2019.04.070.
15. van Smaalen TC, Beurskens DM, Hoogland ER, Winkens B, Christiaans MH, Reutelingsperger CP, van Heurn LW, Nicolaes GA. Presence of Cytotoxic Extracellular Histones in Machine Perfusate of Donation After Circulatory Death Kidneys. *Transplantation.* 2017 Apr;101(4):e93–e101. doi: 10.1097/TP.0000000000001590. PMID: 27906828; PMCID: PMC7228581.
16. Jin Y, Sun M, Lv X, Wang X, Jiang G, Chen C, Wen Z. Extracellular histones play a pathogenic role in primary graft dysfunction after human lung transplantation. *RSC Adv.* 2020;10: 12485–91. DOI: 10.1039/d0ra00127A.
17. Liu T, Huang W, Szatmary P, Abrams ST, Alhamdi Y, Lin Z, et al. Accuracy of circulating histones in predicting persistent organ failure and mortality in patients with acute pancreatitis. *Br J Surg.* 2017 Aug;104(9):1215–1225. doi: 10.1002/bjs.10538. Epub 2017 Apr 24. PMID: 28436602; PMCID: PMC7938821.
18. Hoeksema M, van Eijk M, Haagsman HP, Hartshorn KL. Histones as mediators of host defense, inflammation and thrombosis. *Future Microbiol.* 2016;11(3):441–53. doi: 10.2217/fmb.15.151. Epub 2016 Mar 4. PMID: 26939619; PMCID: PMC5549641.
19. Nomura K, Miyashita T, Yamamoto Y, Munesue S, Harashima A, Takayama H, Fushida S, Ohta T. Citrullinated Histone H3: Early Biomarker of Neutrophil Extracellular Traps in Septic Liver Damage. *J Surg Res.* 2019 Feb;234:132–138. doi: 10.1016/j.jss.2018.08.014. Epub 2018 Oct 10. PMID: 30527465.

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