J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 47(3): 957-966, 2023
Doi: 10.33483/jfpau.1247781

ORIGINAL ARTICLE / ÖZGÜN MAKALE

PREDICTIVE BIOMARKERS OF LIVING DONOR LIVER TRANSPLANTATION

CANLI DONÖR KARACİĞER NAKLİNİN ÖNGÖRÜSEL BİYOBELİRTEÇLERİ

Ömer Faruk ÇİFTÇİ[1], Tevfik Tolga ŞAHİN[2], Hande YÜCE[1], Sezai YILMAZ[2], Neşe Başak TÜRKMEN[1], Şeyma YAŞAR[3], Tülay ÇOBAN[4], Songül ÜNÜVAR[1]*

[1]İnönü University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, 44000, Malatya, Türkiye

[2]İnönü University, Faculty of Medicine, Liver Transplantation Institute, Department of Surgery, 44000, Malatya, Türkiye

[3]İnönü University, Faculty of Medicine, Department of Biostatistics and Medical Informatics, 44000, Malatya, Türkiye

[4]Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, 06560, Ankara, Türkiye

ABSTRACT

Objective: *Liver transplantation using a living donor (LDLT) is currently the most popular method used in the worldwide. Appropriate biomarkers that predict graft status should be used to detect early post-transplant complications that may lead to a rejection reaction.*
Material and Method: *The study involved a total of 44 liver recipients and 44 liver donors, from whom preoperative blood samples were taken and immunoassay and spectrophotometric studies were carried out. The levels of serum neopterin, interferon-gamma (IFN- y), indoleamine-2,3 dioxygenase (IDO), and -glutathione S transferase (a-GST) were assessed using an enzyme-linked immunosorbent test. The activity of glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PD) in erythrocytes was measured using spectrophotometry.*
Result and Discussion: *"Neopterin, IDO, and G6PD levels were significantly higher in the recipient group than in the donor group. The differences in gender and blood groups were statistically insignificant. The rejection reaction developed in 25% of patients and none survived. These findings may facilitate the identification of novel predictive biomarkers for the diagnosis of*

* **Corresponding Author / SorumluYazar:** Songül Ünüvar
e-mail / e-posta: songul.unuvar@inonu.edu.tr, **Phone / Tel.:** +905062454054

Submitted / Gönderilme : 05.02.2023
Accepted / Kabul : 18.08.2023
Published / Yayınlanma : 20.09.2023

acute rejection reactions after LDLT. The clinical use of novel non-invasive biomarkers may provide time and cost advantages.
Keywords: *α-GST liver transplantation, G6PD, IFN-γ, indoleamine-2,3-dioxygenase*

ÖZ

Amaç: *Canlı donör karaciğer nakli (CDKN) şu anda dünya çapında en yaygın karaciğer nakli yaklaşımıdır. Rejeksiyon reaksiyonuna yol açabilecek erken nakil sonrası komplikasyonları tespit etmek için greft durumunu öngören uygun biyobelirteçler kullanılmalıdır.*
Gereç ve Yöntem: *Çalışmaya toplam 44 karaciğer alıcısı ve 44 karaciğer donörü dahil edildi, bunlardan ameliyat öncesi kan örnekleri toplandı, immünoassay ve spektrofotometrik analizler yapıldı. Serum neopterin, interferon gama (IFN-y), indolamin-2,3-dioksijenaz (IDO) ve alfa-Glutatyon S- transferaz (a-GST) seviyeleri, enzime bağlı bir immünosorbent deneyi kullanılarak ölçüldü. Eritrositlerdeki glutatyon redüktaz (GR) ve glukoz-6-fosfat dehidrogenaz (G6PD) aktivitesi spektrofotometri ile ölçüldü.*
Sonuç ve Tartışma: *Neopterin, IDO ve G6PD düzeyleri alıcı grupta donör grubuna göre anlamlı olarak yüksekti. Cinsiyet ve kan gruplarındaki farklılıklar istatistiksel olarak anlamsızdı. Hastaların %25'inde reddetme reaksiyonu gelişti ve hiçbiri hayatta kalmadı. Bu bulgular, CDKN sonrası akut rejeksiyon reaksiyonlarının teşhisi için yeni prediktif biyobelirteçlerin tanımlanmasını kolaylaştırabilir. Yeni invaziv olmayan biyobelirteçlerin klinik kullanımı, zaman ve maliyet avantajları sağlayabilir.*
Anahtar Kelimeler: *α-GST karaciğer nakli, G6PD, IFN-γ, indolamin-2,3-dioksijenaz*

INTRODUCTION

Liver transplantation (LT) is currently the just treatment method recommended for patients with developed liver disease, which is life-threatening and cannot be treated by other methods. Patients may develop post-transplant complications associated with immunosuppressive therapy, such as rejection, infection, hypertension, and malignancy [1]. Liver transplantation involves the replacement of diseased liver tissue with a portion of liver tissue with normal functions harvested from a brain-dead or healthy living person. One of the most common complications after liver transplantation is acute rejection, the most common form of which is cell-mediated rejection, which occurs through the recognition of recipient T lymphocytes and the presentation to donor alloantigens by antigen-presenting cells. Acute rejection must be identified as soon as feasible in order to use an effective anti-rejection treatment and to retain the graft's functionality and integrity [2,3]. Liver transplant process can be assessmented by biochemical tests, such as total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), leukocytosis and eosinophilia, although usual laboratory tests are non-specific and are unsuitable for the effective and timely diagnosis of acute rejection. Liver biopsy, an invasive procedure, can be performed in the event of suspected acute rejection, although the procedure is associated with severe complications. Non-invasive biomarkers include cytokines in saliva, urine, peripheral blood, or other body fluids, or cell surface proteins of various immune cells. As the assessment of such diagnostic biomarkers is not invasive, they have been investigated by researchers for many years as a possible replacement for liver biopsy [4,5].

Neopterin is a chemical of the pyteridine group that is activated by interferon-gamma (IFN-γ), which is made by monocytes, macrophages, dendritic cells, and endothelial cells and released by functional protected T cells. It's been employed as a marker to evaluate cellular consumption [6,7]. Indoleamine 2,3 dioxygenase (IDO) is a cytosolic, heme-containing enzyme involved in the first step of tryptophan catabolism, and has been associated with multiple pathophysiological conditions, including autoimmune disorders, fetomaternal tolerance, cancer, and infectious diseases. It inhibits the proliferation of both T lymphocytes and pathogens by exhausting tryptophan, an essential amino acid, and is expressed by antigen-presenting cells induced by proinflammatory cytokines such as interferon-γ, and its main function is immunosuppressive activity. Interferon-gamma is a cytokine with antiviral, antitumor and immunomodulatory effects, which is crucial for regulations of both congenital and adaptive immune responses [6-9].

IFN-γ is also the strongest inducer of IDO [8,9]. Due to its lower molecular weight and shorter half-life, -glutathione S transferase (α-GST) is a more sensitive biomarker of liver function than common liver tests AST and ALT. α-GST levels were found to be fairly high in patients with acute liver failure and significantly greater in liver transplant recipients who experienced moderate to severe rejection compared to those who did not [10,11]. Glutathione reductase (GR) the main enzyme of the glutathione metabolism converts oxidized glutathione (GSSG), a product of the reactions catalyzed by glutathione S-transferase and glutathione peroxidase to reduced glutathione (GSH). It has an antioxidant effect and is a highly specific marker of liver injury [12,13]. Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme that is critical to glucose metabolism and that protects red blood cells from oxidative stress. Nevertheless, researchers define G6PD as a contraindication for liver donation [14].

This study's objective is to contrast numerous protein biomarkers with normal biochemical tests in order to assess their effectiveness as indicators of acute rejection in living donor liver transplants.

MATERIAL AND METHOD

Participants

This prospective cross-sectional study was conducted by the Turgut Özal Medical Center Liver Transplant Institute between January 2018 and January 2020 (Figure 1). The study groups included 44 liver tissue recipients admitted to the transplant institute and 44 tissue donors who donated tissue for these patients. Patients in the liver tissue recipient group were 30 male and 14 female, whereas those in the liver tissue donor group were 27 male and 17 females. The ethics committee of İnönü University Malatya gave its approval for the study, which was carried out in compliance with the Declaration of Helsinki's tenets (Approval No: 2018/144). All participants gave their official approval for the use of their medical data for research. Peripheral venous blood samples from each participant in the trial were taken and put in typical biochemistry tubes for evaluation. The separated sera were put in tubes and kept at -80°C until analysis after the blood samples were centrifuged at 3,500 rpm for 15 minutes at room temperature. The evaluated parameters included neopterin, IFN-γ, IDO, G6PD, GR, a model for end-stage liver disease (MELD) score, total bilirubin, direct bilirubin, hemoglobin, white blood cells (WBCs), platelets, C-reactive protein (CRP), international normalized ratio (INR), creatinine, albumin, sodium, recipient's age, gender and blood group, rejection rate, operation outcome, donor's age, gender and blood group, diagnosis, and serum levels of ALT, AST, gamma-glutamyl transferase (GGT), ALP and α-GST.

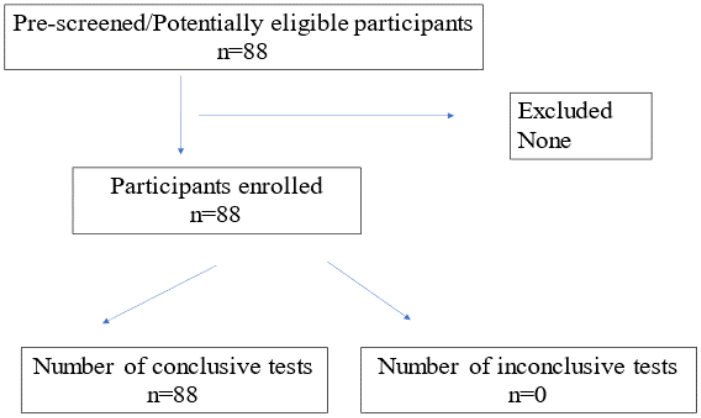

Figure 1. Participant flow diagram

Measurement of Glucose 6-phosphate Dehydrogenase Activity in Erythrocytes

The Beutler method, which relies on the reduction of $NADP^+$ by G6PD in the presence of glucose 6-phosphate, was used to quantify the activity of G6PD in a spectrophotometer at 37°C. The rate of

NADPH synthesis, which is dependent on G6PD activity, was calculated using the rise in absorbance at 340 nm [15].

Measurement of Glutathione Reductase Activity in Erythrocytes

Glutathione reductase activity was measured based on the principle of maximum absorbance of reacting NADPH at 340 nm. The reaction catalyzed by GR results in a decrease in NADPH, and this decrease was monitored spectrophotometrically at 340 nm to determine the enzyme activity [16].

Measurement of Serum Neopterin, IFN-γ, IDO, and α-GST Levels

The serum concentrations of neopterin, IFN-γ, IDO, and α-GST were measured using commercial enzyme-linked immunosorbent assay kits (E3155Hu, ELH-IFNg-1, E0796Hu, and EK1625; Bioassay Technology Laboratory, Ltd.). Serum neopterin, IFN-γ, IDO, and α-GST test measurement ranges were 0.1-38.0 nmol/l, 15-15000 pg/ml, 0.3-90 ng/ml, and 156-10000 pg/ml, respectively. The intra- and inter-assay accuracy coefficients of variation for all ELISA kits were less than 10% and less than 8%, respectively.

Clinical Significance

In the current study, we investigate the non-invasive diagnostic biomarkers for allograft rejection in liver transplant recipients and evaluate the accuracy of these biomarkers in predicting acute rejection by contrasting them with standard biochemical assays used in clinical practice. This is the first study that, to the best of our knowledge, compares the effectiveness of novel protein biomarkers with the outcomes of standard tests. The study's prospective cohort design is another asset. The recipients' serum concentrations of neopterin, IDO, and G6PD were found to be significantly elevated. The creation of novel LDLT diagnostic biomarkers might be made simpler as a result of our findings.

Statistical Analysis

According to power calculations, each group would require at least 21 participants if the biggest neopterin difference between them was 1.4 nmol/l (standard deviation, 0.7 nmol/l), the type I error was 0.05, and the type II error was 0.20. The median (interval) or mean SD is used to present data. The Shapiro-Wilk test was utilized to assess the data's normality. The analysis made use of the independent two-sample t test, Mann-Whitney U test, Pearson 2 test, Yates corrected 2 test, and Fisher exact 2 test where needed. In order to assess the diagnostic performance and the best cutoff values for the variables of interest, a receiver operating characteristic (ROC) curve analysis was carried out. The Biostatistics Department of Inonu University created the web-based DTROC program. The web-based DTROC application, developed by the Biostatistics and Medical Informatics Department of İnonu University Faculty of Medicine, was utilized for the ROC analysis. The correlations between the variables were assessed using the Spearman rank correlation coefficient of 11. P values under 0.05 were regarded as significant. The analyses were done using SPSS version 25 (IBM Corp., Armonk, NY, USA). To calculate odds ratios (ORs), multivariate logistic regression analysis was used.

RESULT AND DISCUSSION

The 44 individuals in the control group had an average age of 32.82 + 8.12 years. Males made up the majority of the contributors. An assessment of blood-type distribution revealed that O(+) was the most common, followed by A(+) in the control group (Table 1). The patient group's 44 participants had a mean age that was higher than that of the control group (46.55 18.82 years). Most tissue recipients were male, similar to the donor group. An assessment of blood-type distribution revealed A(+) to be the most common, followed by O(+) in the patient group (Table 2).

The difference between the two groups median ages was statistically significant ($p<0.001$). While CRP levels were greater in the donor group ($p=0.005$), levels of neopterin, direct bilirubin, AST, ALT, GGT, and total bilirubin were also considerably higher in the patient group ($p<0.001$ for all). Additionally, the donor groups showed significantly higher IDO and G6PD activity ($p=0.028$ for both). Both study groups' GR activity was comparable ($p=0.006$). The patient group's hemoglobin, platelet,

and albumin levels were all lower than those of the control group ($p<0.001$, for all three). Differences in blood type and gender had no impact on the variables under investigation (Table 3). The predictive significance of biomarkers for liver transplants is demonstrated by ROC analysis (Table 4).

Table 1. Demographic and clinical characteristics of the control group

Characteristics		**Liver tissue donors (control group, n=44)**
Age, years		32 (21-50)
Gender	**Female**	17 (38.6)
	Male	27 (61.4)
Blood group	**O (+)**	19 (43.2)
	A (+)	16 (36.4)
	B (+)	8 (18.2)
	AB (+)	1 (2.3)

Values are given as median (minimum-maximum), and number (percentage).

Table 2. Demographic and clinical characteristics of the study group

Characteristics		**Liver tissue recipient (study group, n=44)**
Age, years		50.0 (3.0-72.0)
Gender	**Female**	14 (31.8)
	Male	30 (68.2)
Blood group	**O (+)**	12 (27.3)
	A (+)	22 (50.0)
	B (+)	8 (18.2)
	AB (+)	2 (4.5)
MELD score		23.02±5.08
Diagnosis	**Hepatitis**	14 (31.8)
	Cirrhosis	14 (31.8)
	Liver failure	16 (36.4)
Rejection	**Positive**	11 (25)
	Negative	33 (75)
Operation result	**Live**	33 (75)
	Death	11 (25)

Values are given as median (minimum-maximum), mean ± SD, and number (percentage)

Table 3. Comparison of patient characteristics, and protein biomarker levels in the control and study groups (n=88)

	Group		**P-value[a]**
	Liver tissue donors (control group, n=44)	**Liver tissue recipients (study group, n=44)**	
	Median (Min-Max)	**Median (Min-Max)**	
Age (year)	32 (21-50)	50 (3-72)	**<0.001**
Total Bilirubin (mg/dl)	1.45 (0.42-3.6)	5.45 (1.57-18.07)	**<0.001**
Direct Bilirubin (mg/dl)	0.59 (0.15-1.83)	2.6 (0.77-11.32)	**<0.001**
INR	1.05 (0.85-2.08)	1.95 (1.05-9.86)	**<0.001**
Creatinine (mg/dl)	0.8 (0.5-1.23)	0.83 (0.39-2.05)	0.646
AST (U/L)	170 (14-392)	461.5 (41-3133)	**<0.001**
ALT (U/L)	188 (15-522)	370 (28-2434)	**<0.001**
GGT (U/L)	20 (6-76)	54.5 (15-837)	**<0.001**

Table 3 (*continue*). Comparison of patient characteristics, and protein biomarker levels in the control and study groups (n=88)

		Group		P-value[a]
		Liver tissue donors (control group, n=44)	Liver tissue recipients (study group, n=44)	
		Median (Min-Max)	Median (Min-Max)	
ALP (U/L)		61.5 (30-200)	70 (25-655)	0.317
Hemoglobin (g/dl)		13.9 (10-18.6)	10.55 (5.2-18.1)	**<0.001**
WBC (/L)		19.3 (6.97-30.2)	16 (3.13-65.9)	0.185
Platelets (/L)		244.5 (14-425)	113 (25-1294)	**<0.001**
CRP (mg/l)		0.32 (0.3-6.35)	0.53 (0.3-6.82)	**0.005**
α-GST (µg/l)		0.22 (0.13-2.99)	0.19 (0.05-2.89)	0.324
Neopterin (nmol/l)		0.36 (0.27-2.91)	3.14 (0.31-8654)	**<0.001**
IFN-γ (pg/ml)		0.12 (0.02-2.81)	0.16 (0.02-2.78)	0.126
IDO (ng/ml)		0.4 (0.17-1.76)	0.47 (0.05-1231)	**0.028**
G6PD (units/g/Hgb)		0.02 (0.02-0.03)	0.03 (0.02-0.03)	**0.028**
GR (EU/ml)		0.05 (0.04-0.06)	0.05 (0.04-0.05)	**0.006**
		Mean ± SD	**Mean ± SD**	
Sodium (mEq/l)		136.77±1.93	135.80±3.27	0.058
Albumin (g/dl)		3.32±0.42	2.41±0.65	**<0.001**
		Number (Percent)	**Number (Percent)**	
Gender	**Male**	17 (38.6)	14 (31.8)	0.656
	Female	27 (61.4)	30 (68.2)	
Blood group	**O (+)**	19 (43.2)	12 (27.3)	0.414
	A (+)	16 (36.4)	22 (50.0)	
	B (+)	8 (18.2)	8 (18.2)	
	AB (+)	1 (2.3)	2 (4.5)	

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; G6PD, glucose-6-phosphate dehydrogenase; GGT, gamma-glutamyl transferase; GR, glutathione reductase; GST, glutathione-S-transferase; IDO, indoleamine 2,3-dioxygenase; IFN-γ, interferon-gamma; INR, international normalized ratio; WBC, white blood cells. [a]Values are given as median (minimum-maximum), mean ± SD, or number (percentage). a: Bold values show P <0.05

Table 4. ROC analysis shows the predictive value of biomarkers for liver transplants

Variables	Cutoff	Sensitivity	Specificity	LR+	LR−	PPV	NPV	AUC (95% CI)	*P* value[a]
Age (year)	39.5	72.7 (0.56-0.84)	79.5 (0.30-0.93)	3.56	0.34	78.0	74.5	0.79 (0.68-0.89)	<0.001
Total bilirubin (mg/dl)	2.77	0.95 (0.84-1.00)	0.89 (0.51-0.98)	8.40	0.051	89.4	95.1	0.98 (0.95-1.00)	<0.001
Direct bilirubin (mg/dl)	1.19	0.93 (0.73-1.00)	0.98 (0.85-1.00)	41.00	0.070	97.6	93.5	0.98 (0.97-1.00)	<0.001
INR	1.28	0.91 (0.78-0.98)	0.91 (0.78-0.98)	10.00	0.10	90.9	90.9	0.96 (0.90-0.99)	<0.001
Sodium (mEq/l)	134.5	0.37 (0.23-0.52)	0.89 (0.75-0.96)	3.20	0.72	76.2	58.2	0.62 (0.51-0.72)	0.058
Creatinine (mg/dl)	0.95	0.30 (0.17-0.45)	0.84 (0.70-0.93)	1.86	0.84	65.0	54.4	0.53 (0.41-0.65)	0.652
AST (U/L)	289.5	0.80 (0.65-0.90)	0.93 (0.81-0.99)	11.33	0.24	91.9	80.4	0.88 (0.79-0.94)	<0.001
ALT (U/L)	250.0	0.82 (0.67-0.92)	0.77 (0.62-0.88)	3.60	0.24	78.3	81.0	0.84 (0.74-0.91)	<0.001
GGT (U/L)	41.5	0.64 (0.48-0.78)	0.89 (0.75-0.96)	5.60	0.41	84.8	70.9	0.81 (0.71-0.89)	<0.001

Table 4 (*continue*). ROC analysis shows the predictive value of biomarkers for liver transplants

Variables	Cutoff	Sensitivity	Specificity	LR+	LR−	PPV	NPV	AUC (95% CI)	*P* value[a]
ALP (U/L)	77.5	0.48 (0.33-0.63)	0.80 (0.65-0.90)	2.33	0.66	0.0	60.3	0.56 (0.45-0.69)	0.336
Albumin (g/dl)	2.75	0.68 (0.52-0.81)	0.95 (0.85-0.99)	15.00	0.33	93.8	75.0	0.88 (0.79-0.94)	<0.001
Hemoglobin (g/dl)	11.85	0.66 (0.50-0.80)	0.93 (0.81-0.99)	9.67	0.37	90.6	73.2	0.81 (0.71-0.88)	<0.001
WBC (/L)	14.75	0.48 (0.33-0.63)	0.82 (0.67-0.92)	2.63	0.64	72.4	61.0	0.58 (0.47-0.69)	0.197
Platelets (/L)	185.0	0.84 (0.70-0.93)	0.91 (0.78-0.97)	9.25	0.18	90.2	85.1	0.85 (0.76-0.92)	<0.001
CRP (mg/l)	0.372	0.61 (0.45-0.76)	0.68 (0.52-0.81)	1.93	0.57	65.9	63.8	0.67 (0.56-0.77)	0.003

AUC, area under the curve; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; GGT, gamma-glutamyl transferase; INR, international normalized ratio; LR−, negative likelihood ratio; LR+, positive likelihood result; NPV, negative predictive value; PPV, positive predictive value; WBC, white blood cells. a: Bold values show P < 0.05

Following LT, the recipient's liver receives the donor's intrinsic bilirubin metabolism, which includes heme oxygenation and bilirubin glucuronidation [17]. In the present study, both the total bilirubin and direct bilirubin levels of the donors were below the cut-off points of 2.77 mg/dl and 1.19 mg/dL, respectively. Liver biochemical tests such as GGT, ALT, AST, ALP, and INR are major and sensitive predictors of acute rejection episodes. A previous study identified liver activity tests such as AST/ALT, GGT, ALT, and AST as sensitive predictors of acute rejection in a group of patients receiving liver transplants from living donors, although these enzymes were not associated with the severity of acute rejection [18]. Our study found INR, AST, ALT, GGT, and ALP levels in the donors to be below the cut-off points. A prevalent ailment among individuals who suffer from liver disease is anemia. Despite the high prevalence of anemia among kidney, heart, and lung transplant recipients, little is known regarding the occurrence, progression of anemia after LT, and available treatments. Depending on the criteria used to define anemia, the reported incidence of anemia following LT ranges from 4.3% to 28.2% [19]. In the present study, the hemoglobin levels of the donors were above the cut-off point, whereas the hemoglobin levels of the recipients were both below the cut-off point and below the levels of the donors. Generally, the cause of anemia is not identified and a multifactorial mechanism has been suggested. The most common cause of unexplained anemia may be immune-suppressive medication-induced bone marrow suppression. Experimental and clinical studies have identified a dual role of platelets in liver transplant patients, causing both beneficial and harmful effects. Recent studies have shown that, despite the fact that a low platelet count is usually thought to be a risk factor for perioperative bleeding, platelet aggregation in patients with cirrhosis may not be as poor as previously thought [20]. In the current study, thrombocytopenia was found in a few of the recipients, and we discovered that the recipient group's platelet count was 53.8% lower than the donor group's, which may be due to hemodilution, immune responses, or platelet sequestration in the liver graft after reperfusion. Inflammatory markers are crucial for predicting the prognosis of several disorders, such as cirrhosis and hepatocellular cancer, as well as the mortality rate following liver transplantation and the effectiveness of the procedure. A important indicator of post-transplant mortality is CRP, which is assessed prior to liver transplantation [21]. In the current investigation, we founded that the receivers' CRP level was 65.6% greater than the donors.

Two crucial plasma proteins, albumin and fibrinogen levels, are measured for the monitoring of liver function following liver transplantation. Albumin levels were 12.6% below the cut-off limit in the current investigation [22].

After a significant correlation between neopterin levels and organ rejection following organ transplants, such as kidney, liver, heart, and lung, neopterin was regarded as an important marker in organ transplant patients [23]. A study involving kidney transplant patients found neopterin levels to be elevated in patients with irreversible organ rejection and reached such high levels as 500–1000 nmol/l

in six patients [24]. Another study investigated serum neopterin levels in heart transplant patients, and found low neopterin levels in patients with stable organ functions, while those with organ rejection had significantly increased levels of neopterin [25]. Neopterin serum levels were found to be considerably higher 10 days after transplantation in patients with bacteremia in a study looking at the relationship between postoperative serum neopterin levels and post-transplant septicaemia and death in liver tissue recipients [26].

In a study, it was discovered that blood IFN- γ levels dramatically increased 3 days after transplantation, notably in allograft recipients, and that graft life was also markedly prolonged. It is therefore believed that an important link exists between the IFN-γ producing natural killer (NK) cells and the innate and adaptive immune response immediately after transplantation. IFN- γ is produced immediately after transplantation and its serum levels peak 3 days after transplantation. In the same study, host-produced NK cells, and to a much lesser extent, donor NK cells, have been shown to be the source of most of the IFN- γ produced in the early post-transplantation period. It has further been shown that serum IFN- γ levels are significantly reduced and graft survival is significantly prolonged in the absence of NK cells [27]. Karahanova et al. reported that IFN-γ levels could be used as a significant parameter in the earliest preoperative period in patients undergoing liver transplantation [28]. The control of the immunological response depends on IDO [29]. Recent studies suggest that IDO may have a substantial immunomodulatory role in a range of events, including allergies, tumor immunology, autoimmunity, HIV infection, and transplant immunity [30]. Serum IDO activity was evaluated 30 days after heart transplantation as a part of a study looking into the impact of IDO on rejection. It was discovered that individuals with acute rejection had considerably higher IDO activity than patients without acute rejection [31]. One group of researchers found that kidney transplant recipients had significantly greater IDO levels than the control group [32]. Following renal transplantation, Kaden et al. investigated at kynurenine levels in relation to IDO activity and discovered elevated kynurenine levels in patients who were experiencing acute rejection [33]. The authors thus concluded these levels could serve as a reliable diagnostic tool in the early period. In the present study, a comparison of the IDO levels of the liver transplant recipients and the control group revealed higher IDO levels in the recipients than in the donors.

As GSTs are cytosolic enzymes found inside of cells, serum GST levels are a stronger indicator of cell damage than protein expression. Due to the short half-life of GST (90 min), variations in GST levels are strongly correlated with ongoing liver cell death. Particularly in cases of acute liver failure, α-GST is highly elevated. Furthermore, compared to patients who experience mild or no rejection following liver transplantation, patients who experience moderate to severe post-LT rejection also have considerably higher α-GST levels. As an additional finding, α-GST is a strong correlation with ALT,

AST, and bilirubin. Despite reports that α -GST is a hallmark of acute cellular rejection and a sensitive indicator of liver damage, it has been proposed that α -GST is useless and unspecific as a marker for the diagnosis and treatment of tissue rejection [34]. When our study results were evaluated, neopterin and IDO levels increased in liver tissue recipients due to the activation of the cellular immune system. This immune activation underlying rejection reactions in tissue recipients is expected. Due to the stimulation of the manufacture of this enzyme, which shields red blood cells from oxidative stress, G6PD levels are higher in tissue recipients compared to donors. As a result, while the cellular immune system is activated as a result of liver transplantation, an increase in the level of protective enzymes has been observed with a feedback mechanism against liver damage.

ACKNOWLEDGEMENTS

The Department of Scientific Research Projects at İnönü University provided funding for the present study (Project number: TDK-2019-1641).

AUTHOR CONTRIBUTIONS

Concept: T.T.Ş., H.Y., S.Y., T.Ç., S.Ü.; Design: Ö.F.Ç., S.Ü.; Control: T.T.Ş., Ş.Y., S.Ü.; Sources: T.T.Ş., S.Y., S.Ü.; Materials: T.T.Ş., H.Y., S.Y., N.B.T., S.Ü.; Data Collection and/or

Processing: Ö.F.Ç., Ş.Y., S.Ü.; Analysis and/or Interpretation: H.Y., N.B.T., Ş.Y., S.Ü.; Literature Review: S.Y., T.Ç., S.Ü.; Manuscript Writing: Ö.F.Ç., S.Ü.; Critical Review: S.Y., T.Ç., S.Ü.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

This study was approved by the İnönü University Malatya Clinical Research Ethics Committee (Approval No: 2018/144).

REFERENCES

1. Rai, R. (2013). Liver transplantatation- an overview. The Indian Journal of Surgery, 75(3), 185-191. [CrossRef]
2. Wertheim, J.A., Petrowsky, H., Saab, S., Kupiec-Weglinski, J.W., Busuttil, R.W. (2011). Major challenges limiting liver transplantation in the United States. American Journal of Transplantation, 11(9), 1773-1784. [CrossRef]
3. National Library of Medicine Web site. (2023). Retrieved 2022 May 9, from https://www.ncbi.nlm.nih.gov/books/NBK553074/. Access date: 22.10.2022.
4. Florman, S., Miller, C.M. (2006). Live donor liver transplantation. Liver Transplantation, 12(4), 499-510. [CrossRef]
5. Appenzeller-Herzog, C., Hartleif, S., Vionnet, J. (2021). Clinical parameters and biomarkers predicting spontaneous operational tolerance after liver transplantation: A scoping review. American Journal of Transplantation, 21(10), 3312-3323. [CrossRef]
6. Ünüvar, S., Aslanhan, H., Tanrıverdi, Z., Karakuş, F. (2018). The relationship between neopterin and hepatitis B surface antigen positivity. Pteridines, 29(1),1-5. [CrossRef]
7. Ünüvar, S., Tanrıverdi, Z., Aslanhan, H. (2018). Potential prognostic role of ımmune system activation marker neopterin in patients with type 2 diabetes. Journal of Medical Biochemistry, 37(4), 465-469. [CrossRef]
8. Tanrıverdi, Z., Meteroglu, F., Yüce, H., Şenyiğit, A., Işcan, M., Unüvar, S. (2021). The usefulness of biomarkers in diagnosis of asbestos-induced malignant pleural mesothelioma. Human and Experimental Toxicology, 40(11), 1817-1824. [CrossRef]
9. Mendoza, J.L., Escalante, N.K., Jude, K.M., Sotolongo Bellon, J., Su, L., Horton, T.M., Tsutsumi, N., Berardinelli, S.J., Haltiwanger, R.S., Piehler, J., Engleman, E.G., Garcia, K.C. (2019). Structure of the IFNγ receptor complex guides design of biased agonists. Nature, 567(7746), 56-60. [CrossRef]
10. Czuczejko, J., Mila-Kierzenkowska, C., Szewczyk-Golec, K. (2019). Plasma α-Glutathione S-Transferase Evaluation in Patients with Acute and Chronic Liver Injury. Canadian Journal of Gastroenterology and Hepatology, 2019, 5850787. [CrossRef]
11. Jochum, C., Beste, M., Sowa, J.P., Farahani, M.S., Penndorf, V., Nadalin, S., Saner, F., Canbay, A., Gerken, G. (2011). Glutathione-S-transferase subtypes α and π as a tool to predict and monitor graft failure or regeneration in a pilot study of living donor liver transplantation. European Journal of Medical Research, 16(1), 34-40. [CrossRef]
12. Couto, N., Wood, J., Barber, J. (2016). The role of glutathione reductase and related enzymes on cellular redox homoeostasis network. Free Radical Biology Medicine, 95, 27-42. [CrossRef]
13. Villegas, T., Olmedo, C., Muffak-Granero, K., Comino, A., Garrote, D., Bueno, P., Ferrón, J.A. (2012). Perioperative levels of glutathione reductase in liver transplant recipients with hepatitis C virus cirrhosis. Transplantation Proceedings, 44(6), 1542-1544. [CrossRef]
14. Goralczyk, A.D., Moser, C., Scherer, M.N., Tsui, T.Y., Doenecke, A., Lorf, T., Schlitt, H.J., Obed, A. (2010). Glucose-6-phosphate dehydrogenase deficiency: a contraindication for living donor liver transplantation?. Transplant International, 23(11), e65-e66. [CrossRef]
15. Hunaiti, A.A., Soud, M. (2000). Effect of lead concentration on the level of glutathione, glutathione S-transferase, reductase and peroxidase in human blood. The Science of The Total Environment, 248(1), 45-50. [CrossRef]
16. Carlberg, I., Mannervik, B. (1981). Purification and characterization of glutathione reductase from calf liver. An improved procedure for affinity chromatography on 2',5'-ADP-Sepharose 4B. Analytical Biochemistry, 116(2), 531-536. [CrossRef]

17. Han, S., Yang, J.D., Sinn, D.H., Ko, J.S., Kim, J.M., Shin, J.C., Son, H.J., Gwak, M.S., Joh, J.W., Kim, G.S. (2016). Higher bilirubin levels of healthy living liver donors are associated with lower posttransplant hepatocellular carcinoma recurrence. Transplantation, 100(9), 1933-1938. [CrossRef]
18. Chiu, K.W., Chen, Y.S., de Villa, V.H., Wang, C.C., Eng, H.L., Wang, S.H., Liu, P.P., Jawan, B., Huang, T.L., Cheng, Y.F., Chen, C.L. (2005). Characterization of liver enzymes on living related liver transplantation patients with acute rejection. Hepato-Gastroenterology, 52(66), 1825-1827.
19. Maheshwari, A., Mishra, R., Thuluvath, P.J. (2004). Post-liver-transplant anemia: Etiology and management. Liver Transplantation, 10(2), 165-173. [CrossRef]
20. Pereboom, I.T., Lisman, T., Porte, R.J. (2008). Platelets in liver transplantation: Friend or foe? Liver Transplantation, 14(7), 923-931. [CrossRef]
21. Artz, A.S., Wickrema, A., Dinner, S., Godley, L.A., Kocherginsky, M., Odenike, O., Rich, E.S., Stock, W., Ulaszek, J., Larson, R.A., van Besien, K. (2008). Pretreatment C-reactive protein is a predictor for outcomes after reduced-intensity allogeneic hematopoietic cell transplantation. Biology of blood and marrow transplantation: Journal of the American Society for Blood and Marrow Transplantation, 14(11), 1209-1216. [CrossRef]
22. Amouzandeh, M., Nowak, G., Januszkiewicz, A., Wernerman, J., Rooyackers, O., Norberg, Å. (2018). Albumin mass balance and kinetics in liver transplantation. Critical Care, 22(1), 152. [CrossRef]
23. Fuchs, D., Weiss, G., Reibnegger, G., Wachter, H. (1992). The role of neopterin as a monitor of cellular immune activation in transplantation, inflammatory, infectious, and malignant diseases. Critical Reviews in Clinical Laboratory Sciences, 29(3-4), 307-341. [CrossRef]
24. Schäfer, A.J., Daniel, V., Dreikorn, K., Opelz, G. (1986). Assessment of plasma neopterin in clinical kidney transplantation. Transplantation, 41(4), 454-459. [CrossRef]
25. Müller, T.F., Vogl, M., Neumann, M.C., Lange, H., Grimm, M., Müller, M.M. (1998). Noninvasive monitoring using serum amyloid A and serum neopterin in cardiac transplantation. Clinica Chimica Acta; International Journal of Clinical Chemistry, 276(1), 63-74. [CrossRef]
26. Oweira, H., Lahdou, I., Daniel, V., Hofer, S., Mieth, M., Schmidt, J., Schemmer, P., Opelz, G., Mehrabi, A., Sadeghi, M. (2016). Early post-transplant neopterin associated with one year survival and bacteremia in liver transplant recipients. Human Immunology, 77(1), 115-120. [CrossRef]
27. Obara, H., Nagasaki, K., Hsieh, C.L., Ogura, Y., Esquivel, C.O., Martinez, O.M., Krams, S.M. (2005). IFN-gamma, produced by NK cells that infiltrate liver allografts early after transplantation, links the innate and adaptive immune responses. American Journal of Transplantation, 5(9), 2094-2103. [CrossRef]
28. Karakhanova, S., Oweira, H., Steinmeyer, B., Sachsenmaier, M., Jung, G., Elhadedy, H., Schmidt, J., Hartwig, W., Bazhin, A.V., Werner, J. (2016). Interferon-γ, interleukin-10 and interferon-inducible protein 10 (CXCL10) as serum biomarkers for the early allograft dysfunction after liver transplantation. Transplant Immunology, 34, 14-24. [CrossRef]
29. Yuasa, H.J., Takubo, M., Takahashi, A., Hasegawa, T., Noma, H., Suzuki, T. (2007). Evolution of vertebrate indoleamine 2,3-dioxygenases. Journal of Molecular Evolution, 65(6), 705-714. [CrossRef]
30. Luan, X., Liao, W., Lai, X., He, Y., Liu, Y., Gong, J., Li, J. (2012). Dynamic changes of indoleamine 2,3-dioxygenase of Kupffer cells in rat liver transplant rejection and tolerance. Transplantation Proceedings, 44(4), 1045-1047. [CrossRef]
31. Suarez-Fuentetaja, N., Domenech-Garcia, N., Paniagua-Martin, M.J., Marzoa-Rivas, R., Barge-Caballero, E., Grille-Cancela, Z., Pombo-Otero, J., Muñiz-García, J., Castro-Beiras, A., Crespo-Leiro, M.G. (2012). Indoleamine, 2-3 dioxygenase activity could be an early marker of graft rejection in heart transplantation. Transplantation Proceedings, 44(9), 2645-2648. [CrossRef]
32. Yilmaz, N., Ustundag, Y., Kivrak, S., Kahvecioglu, S., Celik, H., Kivrak, I., Huysal, K. (2016). Serum indoleamine 2,3 dioxygenase and tryptophan and kynurenine ratio using the UPLC-MS/MS method, in patients undergoing peritoneal dialysis, hemodialysis, and kidney transplantation. Renal Failure, 38(8), 1300-1309. [CrossRef]
33. Kaden, J., Abendroth, D., Völp, A., Marzinzig, M. (2015). Dynamics and diagnostic relevance of kynurenine serum level after kidney transplantation. Annals of Transplantation, 20, 327-337. [CrossRef]
34. Ng, K.T., Yeung, O.W., Lam, Y.F., Liu, J., Liu, H., Pang, L., Yang, X.X., Zhu, J., Zhang, W., Lau, M.Y.H., Qiu, W.Q., Shiu, H.C., Lai, M. K., Lo, C.M., Man, K. (2021). Glutathione S-transferase A2 promotes hepatocellular carcinoma recurrence after liver transplantation through modulating reactive oxygen species metabolism. Cell Death Discovery, 7(1), 188. [CrossRef]