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Original Article

Assessment of Hepatic Steatosis Using Clinical and Laboratory Parameters with Computed Tomography Comparison in Living Liver Donor Candidates

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ABSTRACT

Objective: Hepatic steatosis (HS) is a critical element in evaluation of living liver donor candidates (LLDC). We aimed to find predictors of HS while correlating computed tomography (CT) based attenuation assessments with clinical parameters of LLDC.

Methods: A total of 524 LLDC were included in the study. From those, 227 of them were declined due to HS detected by CT and 297 of them underwent successful donation process. These two groups were evaluated statistically in terms of CT based liver attenuation indices, clinical and laboratory parameters to find predictors of HS.

Results: Other than low-density lipoprotein, neutrophil count, neutrophil/lymphocyte ratio, platelet distribution width, platelet count, and alpha fetoprotein, all of the laboratory parameters were different between the groups ($p<0.05$). The median liver attenuation value in accepted donors was 64 and in declined donors, it was 51 ($p<0.001$). Body mass index (BMI) had the highest diagnostic accuracy for HS followed by alanine aminotransferase (ALT). The cutoff value for BMI (72% sensitivity) was 26.3 kg/m^2 and it was 21 IU/L for ALT (69% sensitivity).

Conclusion: BMI>26.3 and ALT>21 correlate well with HS and those subjects should be evaluated later if more appropriate donor candidate is present.

INTRODUCTION

Living donor liver transplantation is developed to compensate for low organ donation rates. But this meticulous procedure requires thorough evaluation to minimize the risk of complications in both donor and recipient.

One of the critical elements for donor selection is degree of hepatic steatosis (HS) in donor liver. Because, rate of HS determines both graft function in recipient and recovery of donor. According to the Inonu University Liver Transplantation Institute data, majority of the donors were rejected due to small remnant size (43%) and fatty changes of liver (38%).[1] By inference, fat accumulation is a common abnormality of liver and frequent reason for donor refusal. At present, liver biopsy is the reference standard method for the quantitative evaluation of HS. However, there are drawbacks to this method. Because only small part of liver can be assessed and interobserver variability can cause misjudgments.[2,3] There can be also some complications which have been reported to be lower than 1% such as parenchymal bleeding and bile leakage.[4] Still, some centers perform liver biopsy routinely. Yet, obtaining liver biopsies from relatively healthy population is an invasive procedure. In this regard, non-invasive tools have been studied in determination of HS.[5] Ultrasonography, cross-sectional imaging methods, particularly magnetic resonance imaging (MRI), and three-dimensional multi-slice computed tomography (MSCT) are emerging as the reference standard of choice. MSCT is not complex as MRI and performs better than ultrasonography especially for macrosteatosis 30% or over.[6] It is also widely available across transplantation centers. At the institute, currently,

HS is assessed on MSCT using attenuation difference between liver and spleen in the step 2 of routine liver donor evaluation process which was defined by Trotter.[7] If liver attenuation index (LAI) is more than 5, donor is presumed to have 0–5% steatosis and as a second line of imaging, MRI is performed.

Although it is time consuming and not cost effective, at the institute, we perform imaging studies for HS evaluation in all donor candidates. On the other hand, MSCT involves use of ionization radiation. Based on these facts, in this retrospective study, we aimed to find predictors of HS while correlating computed tomography based attenuation assessments with clinical parameters of living liver donor candidates (LLDC). Thus, we could sort potential donors by likelihood of HS derived from this data and step 2 evaluation could be performed in order starting from donor with anticipated less steatosis while reducing costs caused by imaging studies and time lost during donor selection process.

MATERIALS AND METHODS

This retrospective study was conducted at Inonu University Institute of Liver Transplantation after obtaining Inonu University Scientific Research Ethics Committee Approval (2020/765).

At the institute, standard donor selection process consists of three steps.[1] The first phase includes clinical assessment and laboratory tests. If a potential donor is found to be eligible, second phase of evaluation process is initiated which involves MSCT and MRI scans. Imaging studies are used to assess graft and remnant volume, degree of HS, vascular, and biliary anatomy. At the institute, LLDC with macrosteatosis more than 10% are not accepted. In this regard, HS is determined using LAI which is derived from the calculation of difference between mean hepatic and spleen attenuation on MSCT scan. If LAI is >5 and vascular anatomy is suitable for donation, donors undergoes MRI scan for biliary anatomy assessment, HS, and occult disease. After step 2, most appropriate donor is determined by institutional review board and subsequently operation is planned.

For this study, first, clinic database of the institute was reviewed for declined LLDC because of high rate of HS based (LAI <5) on MSCT evaluation between January 2015 and May 2020. Initial search delivered 263 subjects. From those, without access to clinical parameters or imaging studies were excluded and a total of 227 declined donors (group 1) were included in the study. Second, database was searched again for living liver donors underwent successful donation process between January 2015 and May 2020. Initial search delivered 309 subjects. From those, rejected due to intraoperative findings of occult liver disease and those with missing clinical parameters or imaging studies were excluded and a total of 297 donors (Group 2) were enrolled in the study. Totally, 524 subjects whom fulfilled all the criteria were included in the study (Fig. 1).

Standard MSCT scan involves pre-contrast and non-ionic, contrast-enhanced arterial, portal, and hepatic phase thin-slice scanning. For this study, MSCT scans performed in the second phase of operation planning were retrospectively reviewed by the surgeons blinded to the donor selection results and surgical findings on the picture archiving and communication system in a random order. MSCT scans were performed in the all subjects with the same equipment (Somatom Definition, 256×256; Siemens Healthineers, GmbH, Erlangen, Germany). For this study alone, two common types of liver attenuation indices were calculated again for each subject involved in the study. Attenuation of the both organs was measured as Hounsfield units using three circular region of interest (ROI) each measuring 100±5 mm^2 on the same MSCT section. Liver

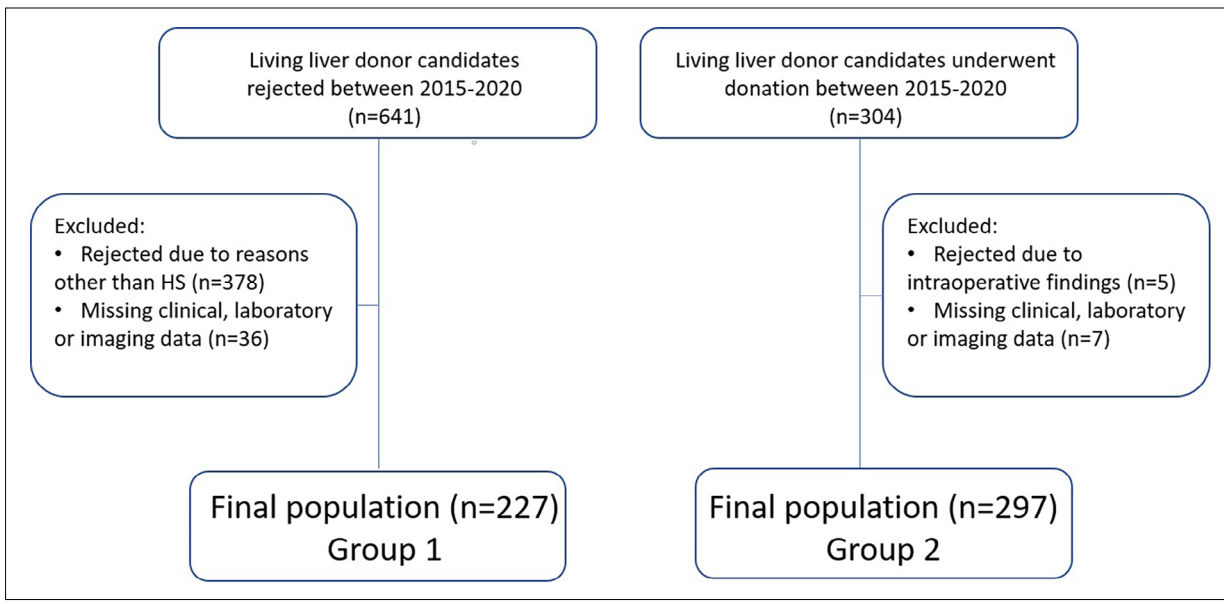

Figure 1. Flow diagram of study subjects.

attenuation was determined by averaging 2 ROI measurements on the right lobe and 1 ROI measurement on the left lobe. Spleen attenuation was determined by averaging upper, middle, and lower pole ROI measurements. Utmost care was taken to leave vessels and other causes of heterogeneity. The two common LAI measurement methods defined in the literature were as follows: (i) The difference between liver and spleen attenuation (CL-S), (ii) the ratio of liver to spleen attenuation (CL/S).[8,9] These density measures took <5 min in the all cases.

In addition to MSCT scans, MRI scans of accepted donors were also obtained. For magnetic resonance, a 3 Tesla scanner (Magnetom, Siemens, Erlangen, Germany) was utilized. Both MRI and magnetic resonance spectroscopy (MRS) were conducted using multi-echo dixon and single-voxel MRS (HISTO) sequences to calculate hepatic proton density fat fraction (PDFF). MRS was performed by HISTO with stimulated echo acquisition mode. After scanning, the system performed postprocessing automatically using LiverLab software package (Siemens Healthcare, Erlangen, Germany). According to the MRS assessments, all of the accepted donors had <5% hepatic PDFF.

For the comparison of the groups and to find predictors of HS, clinical and laboratory parameters of the accepted and declined donors (n=524) were obtained. From the clinical parameters; age, gender, and body mass index (BMI) were used. BMI was calculated as weight (kg)/stature (m2). From the laboratory values; triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL), very-LDL (VLDL), high-density lipoprotein cholesterol (HDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), neutrophil (NE) and lymphocyte (LYM) count, platelet distribution width (PDW), platelet count (PLT), international normalized ratio (INR), and alpha fetoprotein (AFP) levels which were measured by standard laboratory methods after at least 8 h fasting were obtained. Moreover, various non-invasive panels which were developed and tested in the literature to diagnose HS or chronic liver disease such as NE/LYM ratio, ALT/TG ratio, AST/ALT ratio, and AST/PLT ratio were calculated and used in the statistical analysis.[10–13]

Statistical analysis

Statistical analysis was performed on SPSS 23.0 software package (SPSS, Inc., Chicago, IL). Categorical data were expressed as number (percentage) and continuous data as median (interquartile range 25th -75th percentile). For statistical comparison between the groups, Chi-Square test for categorical data and Mann-Whitney U test for continuous data were used. To evaluate the strength of relationship between the variables and LAI measurements, Spearman correlation analysis was used and strength of relationship was reported as correlation coefficient (CC). Receiver operating characteristics (ROC) analysis was

Table 1. Comparison of the clinical and laboratory parameters between the groups

Variables	Accepted donors (n=297)	Declined donors (n=227)	p-value
Age, years	30 (25–35)	37 (31–41)	<0.001
Gender			<0.001
Male	205 (69)	195 (86)	
Female	92 (31)	32 (14)	
BMI, kg/m^2	24.1 (21.8–26.1)	27.7 (26.1–29.9)	<0.001
Male	24.4 (21.9–26.6)	27.6 (26.1–29.7)	<0.001
Female	24.6 (22.6–28.6)	27.3 (24.9–29.9)	0.005
TG, mg/dL	100 (67–143)	141 (86–223)	<0.001
TC, mg/dL	170 (147–191)	177 (154–209)	0.004
LDL, mg/dL	101 (82–121)	101 (83–127)	0.397
VLDL	20 (13–27)	28 (17–44)	<0.001
HDL, mg/dL	44 (37–52)	39 (34–44)	<0.001
AST, IU/L	18 (15–21)	21 (17–25)	<0.001
ALT, IU/L	16 (12–23)	26 (18–38)	<0.001
AST/ALT ratio	1.06 (0.8–1.3)	0.75 (0.59–1)	<0.001
ALT/TG ratio	0.17 (0.11–0.24)	0.19 (0.11–0.3)	0.003
NE, 10^3/μL	4.2 (3.4–5.3)	4.4 (3.5–5.6)	0.132
LYM, 10^3/μL	2.4 (2.1–2.8)	2.5 (2.2–3)	0.004
NE/LYM ratio	1.7 (1.4–2.1)	1.7 (1.3–2.1)	0.959
PDW, %	12 (10.6–13.6)	12 (10.9–13.9)	0.903
PLT, 10^9/L	258 (229–298)	272 (222–307)	0.302
AST/PLT ratio	0.06 (0.05–0.08)	0.07 (0.05–0.1)	<0.001
INR	0.96 (0.93–1)	0.95 (0.91–0.98)	0.005
AFP, ng/mL	1.8 (0.8–3.2)	1.7 (0.8–2.8)	0.680

used to investigate the status of clinical and laboratory variables in diagnosis of more than 5% HS as well as optimal cut-off values to predict HS. For the ROC analysis, the MSCT-derived LAI was used as reference for HS. CL-S <5 was accepted as more than 5% fatty.

RESULTS

A total of 524 LLDC were enrolled in this retrospective study. 297 (57%) of those were approved as liver donors after institutional review board evaluation and underwent successful donation process. On the other hand, 227 (43%) were declined as liver donors due to HS assessed by MSCT in the step 2 of donor selection process. Most of the donor candidates were male and declined donors were older than accepted donors (p<0.001). BMI of declined donors were greater than accepted donors (p<0.001). Other than LDL, NE count, NE/LYM ratio, PDW, PLT and AFP, all of the laboratory parameters were different between the groups (p<0.05) (Table 1).

Liver attenuation values of accepted donors were statistically higher than of declined donors. The median liver attenuation value in accepted donors were 64 (62–67) and in declined donors, it was 51 (45–55). Spleen attenuation values were not statistically different between the groups (p=0.583). LAI values were statistically different between the groups (p<0.001) (Table 2).

Correlation analysis revealed positive relationship between the two types of liver attenuation indices and HDL, INR, and AST/ALT ratio (p<0.001). On the contrary, between the liver attenuation indices and TG, VLDL, AST, ALT, BMI, ALT/TG ratio, and AST/PLT ratio, there was negative correlation (p<0.001). However, those correlations were relatively marginal and the CC of ALT with both CL-S (r=−0.504, p<0.001) and (r=−0.498, p<0.001) CL/S was the highest followed by AST/ALT ratio and BMI. On the other hand, there was no correlation between the liver attenuation indices and TC, PDW, PLT, HDL, and AFP (Table 3).

The ROC analysis suggested that BMI had the highest diagnostic accuracy for HS, with an area under the receiver operating characteristic curve (AUROC) of 0.769. The cutoff value of 26.3 kg/m2 had the highest sensitivity (72%) and specificity (72%) for detecting CL-S <5. The ALT had similar AUROC to BMI with 0.756. Regarding ALT, the cut-

Table 4. The diagnostic accuracy of clinical and laboratory values for the determination of MSCT measured hepatic steatosis

Variables	Cut-off value	AUROC	p-value
TG, mg/dL	113	0.653	<0.001
VLDL, mg/dL	22	0.653	<0.001
AST, IU/L	18	0.663	<0.001
ALT, IU/L	21	0.756	<0.001
HDL, mg/dL	40	0.339	<0.001
INR	0.95	0.405	<0.001
AST/ALT ratio	0.9	0.270	<0.001
BMI, kg/m^2	26.3	0.769	<0.001
ALT/TG ratio	0.18	0.561	0.027
AST/PLT ratio	0.07	0.605	<0.001

Table 2. Comparison of the attenuation values and indices between the groups

Variables	Accepted donors (n=297)	Declined donors (n=227)	p-value
Liver ROI	64 (62–67)	51 (45–55)	<0.001
Spleen ROI	52 (49–54)	52 (49–54)	0.583
CL-S	12 (9–15)	0 (–7–4)	<0.001
CL/S	1.2 (1.1–1.3)	1 (0.8–1)	<0.001

Table 3. Correlations of the liver attenuation indices and clinical and laboratory parameters

Parameters	CL-S		CL/S	
	Correlation coefficient	Sig. (p-value)	Correlation coefficient	Sig. (p-value)
TG	−0.255	<0.001	−0.246	<0.001
VLDL	−0.253	<0.001	−0.244	<.001
AST	−0.329	<0.001	−0.333	<0.001
ALT	−0.504	<0.001	−0.498	<0.001
HDL	0.273	<0.001	0.266	<0.001
INR	0.132	0.002	0.128	0.003
AST/ALT ratio	0.451	<0.001	0.442	<0.001
BMI	−0.424	<0.001	−0.398	<0.001
ALT/TG ratio	−0.146	<0.001	−0.151	<0.001
AST/PLT ratio	−0.249	<0.001	−0.257	<0.001

off value with the highest sensitivity (69%) and specificity (68%) was 21. Other parameters showed less remarkable diagnostic potential than BMI and ALT (Table 4).

DISCUSSION

Liver steatosis is one of the important factors that determine graft function after transplantation. At our institution, the presence of macrosteatosis >10% contraindicates liver transplantation to minimize any potential risks to the recipient. In this regard, unenhanced MSCT is used to predict HS. The method solely based on the study by Limanond et al.[14] in which they reported that CL-S >5 correctly predicted less than 5% macrovesicular steatosis in 25 of 27 patients. Apart from that, we evaluate HS using CL-S >5 criterion during the selection of LLDC. We also validated those MSCT assumptions in accepted donors with MRS-PDDF. All of the accepted donors had <10% PDDF. The MRS-PDDF has been stated as the non-invasive standard of reference for the diagnosis of HS in the 2016 European Association for the Study of the Liver guidelines on non-alcoholic fatty liver disease (NAFLD).[15] Thus, in the last years, MRS-PDDF evaluation of HS has been implemented to the routine practice at the institute.

As aforementioned, submission of multiple donor candidates prolongs preoperative evaluation process as well as time interval between diagnosis and surgery. As we reported before, after small remnant size, fatty liver changes constituted the second main reason to reject 38% of donor candidates.[1] When assessment of multiple donor candidates combined with rejection due to fatty liver, much valuable time is lost along with substantial expense. In this study, we aimed to find predictors of HS by correlating clinical and laboratory parameters with unenhanced MSCT attenuation measurements so that we could be able to sort donors by anticipated HS. Thus, particularly imaging studies would be performed in an order. This could reduce costs and time lost especially during the second step of donor selection process. However, this approach may generate a censurable situation that not only HS but also vascular variants can be a reason for donor rejection whereas nearly 3% of donor candidates were rejected at the institute because of vascular variants. Fatty liver changes are much more prevalent among donor candidates.

In this regard, in addition to standard laboratory values, several parameters easy to calculate were included in the statistical analysis to find predictors of HS. All of the parameters were used previously in various NAFLD or liver fibrosis. For instance; AST/ALT ratio and PLT are components of NAFLD score and NE/LYM ratio was studied as a marker of steatohepatitis by Alkhouri and colleagues.[11] In addition, Simental-Mendia et al.[10] worked with asymptomatic obese women whether ALT/TG ratio could be used as a marker of NAFLD. Furthermore, AST/PLT ratio was proposed as a simple predictor of non-alcoholic steatohepatitis and hepatic fibrosis.[13,16]

When declined donor candidates were compared with accepted donors, lipid profiles other than LDL showed statistically significant difference between the groups. In their study, Hamirani et al.[17] also did not report significant difference for LDL between the subjects with normal liver and with fatty liver. We also did not observe significant difference for PDW, PLT, NE/LYM ratio, and AFP between the groups so that, they were not included in the correlation analysis.

The groups were also different in terms of both LAI values. The median number of CL-S of the accepted donors was 64 which was in line with Kodama et al.'s[18] report which showed that for biopsy-proven 0% hepatic fat, unenhanced MSCT measured liver attenuation was 64.4±3.1 and Pickhardt et al.[19] who showed that attenuation values for normal liver ranged between 60–70 HU. Spleen attenuation values were not different between the groups as expected because spleen is not affected from various pathological processes such as steatosis. Thus, it can serve as a good internal control of steatosis.[20] We also calculated liver to spleen attenuation ratio as an index of HS. The median number of CL/S of the accepted donors was 1.2 which was also in agreement with the literature. In the work of Iwasaki and colleagues, the optimal cutoff to exclude more than moderate steatosis was found to be 1.1.[21]

To clarify the relationship between the clinical and laboratory parameters and MSCT measured LAI values, correlation analysis was performed. It revealed marginal negative relationship between BMI and MSCT measurements. A similar correlation was also found for ALT. Moreover, TG and VLDL were also negatively correlated with LAI values but the relationship was less strong. However, there was no relationship between TC levels and LAI values. According to the ROC analysis, BMI was the most valuable parameter to predict HS followed by ALT. The optimal cutoff value for BMI was 26.3 (Specificity 72%) and for ALT it was 21 (Specificity 69%). Beyond those values, donors were more likely to have HS.

Although fatty infiltration is known to be more prevalent in subjects with high BMI, BMI as a predictive factor of HS was studied by Ryan et al.[22] and they indicated that BMI do not give accurate prediction of hepatic fat. Yet, in the study of Gaba et al.,[23] BMI showed moderate correlation with HS ($r=0.37$, $p<0.001$) and they reported that a BMI ≥32 should raise suspicion for liver steatosis. In another study, Saran et al.[8] found similar correlation between BMI and LAI values as in our study. The CC for BMI and CL-S was −0.506. For BMI and CL/S it was −0.538. In our study, relationship between ALT and LAI values was strongest and diagnostic potential of ALT in HS was similar to BMI. Among the markers, ALT is well known to be specific to liver damage and an indicator of fat accumulation.[24] In the study of Park et al.,[25] ALT was found as an independent risk factor of HS only in men. Furthermore, in another study ALT was found to be independently correlated with hepatic TG contents in obese subjects. Those results have justified use of ALT as a predictor of liver fat. In this study,

especially over 21 IU/L, specificity of ALT to MSCT measured HS was increased.

Our study has some limitations. First, we do not perform liver biopsy in the preoperative evaluation of living liver donors as a routine. Therefore, we could not make correlations between histologic fat quantification and imaging. Second ROI measurements are prone to sampling errors. However, we also performed MRS-PDFF in the final stage of preoperative donor evaluation and indirectly controlled MSCT examinations. However, liver biopsy with a small volume also can not represent the entire liver, because distribution of fat is heterogeneous.

In conclusion, according to the literature, unenhanced MSCT provides high performance especially in the qualitative assessment of macrovesicular steatosis ≥30%.[6] However, we validated MSCT measurements with MRS-PDFF in accepted donors and CL-S <5 as a criterion for HS showed great concordance with PDFF measurements. Non-invasive assessment of LLDC by MSCT provides great comfort. If BMI is over 26.3 and ALT over 21 IU/L, HS should be considered and those subjects should be evaluated later if more appropriate donor candidate is present because those are likely to have HS. Staging donor candidates by clinical and laboratory values as indicated may help transplant surgeons reduce time lost during preoperative arrangements and costs due to multiple imaging studies.

Ethics Committee Approval

This study approved by the İnönü University Clinical Research Ethics Committee (Date: 16.06.2020, Decision No: 2020/765).

Informed Consent

Retrospective study.

Peer-review

Externally peer-reviewed.

Authorship Contributions

Concept: T.S, D.Y.B.; Design: T.S, D.Y.B.; Supervision: T.S, D.Y.B.; Fundings: T.S, D.Y.B.; Materials: T.S, D.Y.B.; Data: T.S, D.Y.B.; Analysis: T.S, D.Y.B.; Literature search: T.S, D.Y.B.; Writing: T.S, D.Y.B.; Critical revision: T.S, D.Y.B.

Conflict of Interest

None declared.

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Canlı Karaciğer Verici Adaylarında Bilgisayarlı Tomografi Karşılaştırması ile Klinik ve Laboratuvar Parametreleri Kullanılarak Hepatik Steatozun Değerlendirilmesi

Amaç: Hepatik steatoz (HS), canlı karaciğer donör adaylarının değerlendirilmesinde kritik bir unsurdur. Bu çalışmada, bilgisayarlı tomografi (BT) tabanlı atenüasyon değerlendirmelerini canlı karaciğer donör adaylarının klinik parametreleriyle ilişkilendirirken karaciğer yağlanmasının öngörücülerini bulmayı amaçladık.

Gereç ve Yöntem: Çalışmaya toplam 524 canlı karaciğer donör adayı dahil edildi. Bunlardan 227'si BT ile tespit edilen HS nedeniyle reddedildi ve 297'si başarılı bağış süreci geçirdi. Bu iki grup, HS'nin öngörücülerini bulmak için BT tabanlı karaciğer atenüasyon indeksleri, klinik ve laboratuvar parametreleri açısından istatistiksel olarak değerlendirildi.

Bulgular: Gruplar arasında düşük yoğunluklu lipoprotein, nötrofil sayısı, nötrofil/lenfosit oranı, trombosit dağılım genişliği, trombosit sayısı ve alfa fetoprotein dışındaki tüm laboratuvar parametreleri farklıydı ($p<0.05$). Kabul edilen vericilerde ortanca karaciğer atenüasyon değeri 64, reddedilen vericilerde ise 51'di ($p<0.001$). Vücut kitle indeksi (BMI), HS için en yüksek tanısal doğruluğa sahipti ve bunu alanin aminotransferaz (ALT) izledi. BMI (%72 duyarlılık) için cut-off değeri 26.3 kg/m^2 ve ALT için 21 IU/L (%69 duyarlılık) idi.

Sonuç: BMI >26.3 ve ALT >21, HS ile iyi koreledir ve daha uygun donör adayı varsa bu denekler daha sonra değerlendirilmelidir.

Anahtar Sözcükler: Canlı bağışçılar; donör seçimi; karaciğer nakli; manyetik rezonans görüntüleme; steatoz; yağlı karaciğer.