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High-Density Lipoprotein Subfraction Changes in End-Stage Liver Failure

Son Dönem Karaciğer Yetmezliğinde Yüksek Yoğunluklu Lipoprotein Subfraksiyon Değişiklikleri

ABSTRACT

Objective:
This work was designed to ascertain high-density lipoprotein (HDL) subfractions and HDL-related enzyme alterations in end stage liver failure (ESLF).

Material and Methods:
Twenty patients with ESLF and twenty control subjects (liver donors) were chosen for the study. Before the transplant, serum samples from each patient and control were evaluated. Continuous disc polyacrylamide gel electrophoresis was performed to determine HDL subfraction changes and ELISA was carried out to determine serum levels of lecithin–cholesterol acyltransferase (LCAT), apolipoprotein A-1 (Apo A1) and cholesteryl ester transfer protein (CETP).

Results:
Liver failure patients had significantly higher levels of triglycerides (TG) and very low-density lipoprotein (VLDL) compared to healthy donors. Additionally, these patients showed significant increases in levels of aspartate aminotransaminase (AST), alkaline phosphatase (ALP), alanine aminotransaminase (ALT), and blood urea nitrogen (BUN), while albumin was notably lower compared to controls. Even though HDL cholesterol concentrations were not considerably altered between the two groups, ESLF patients had a marked raise in the HDL-large subfraction and a significant decline in the HDL-small subfraction in contrast to controls. Moreover, liver failure patients had considerably lower serum Apo A1 levels compared to healthy controls, but there was no significant difference in LCAT and CETP levels between the two groups.

Conclusion:
HDL subfraction profile can distinguish between healthy donors and liver failure patients. The results also indicate that levels of Apo A1, which performs a crucial function in HDL metabolism, are lower in ESLF. This decrease in HDL-small subfractions may be due to impaired anabolism resulting from hepatic failure.

Key Words:
End-stage liver failure, HDL Subfractions, Apo A1, CETP, LCAT

ÖZ

Amaç:

Bu çalışma, son dönem karaciğer yetmezliğinde (ESLF) yüksek yoğunluklu lipoprotein (HDL) alt fraksiyonlarını ve HDL ile ilişkili enzim değişikliklerini belirlemek için tasarlanmıştır.

Gereç ve Yöntemler:

Çalışma için 20 ESLF hastası ve 20 kontrol (karaciğer donörü) kişi seçildi. Nakil öncesinde her hasta ve kontrolden alınan serum örnekleri değerlendirildi. HDL alt fraksiyon değişikliklerini belirlemek için sürekli disk poliakrilamid jel elektroforezi yapıldı ve lesitin-kolesterol asiltransferaz (LCAT), apolipoprotein A-1 (Apo A1) ve kolesteril ester transfer proteininin (CETP) serum seviyelerini belirlemek için ELISA yapıldı.

Bulgular:

Karaciğer yetmezliği olan hastalarda, sağlıklı donörlerle karşılaştırıldığında anlamlı derecede yüksek trigliserit (TG) ve çok düşük yoğunluklu lipoprotein (VLDL) seviyeleri vardı. Ek olarak, bu hastalarda aspartat aminotransaminaz (AST), alkalin fosfataz (ALP), alanin aminotransaminaz (ALT) ve kan üre nitrojeni (BUN) düzeylerinde önemli artışlar görülürken, albümin kontrollere kıyasla belirgin şekilde düşüktü. HDL kolesterol konsantrasyonları iki grup arasında önemli ölçüde değişmese de, ESLF hastalarında kontrollerin aksine HDL-büyük alt bölümünde belirgin bir artış ve HDL-küçük alt bölümünde önemli bir düşüş vardı. Ayrıca karaciğer yetmezliği olan hastaların serum Apo A1 düzeyleri sağlıklı kontrollere göre oldukça düşüktü ancak LCAT ve CETP düzeyleri açısından iki grup arasında anlamlı bir fark yoktu.

Sonuç:

HDL alt fraksiyon profili, sağlıklı donörler ile karaciğer yetmezliği olan hastaları birbirinden ayırabilir. Sonuçlar ayrıca HDL metabolizmasında çok önemli bir işlevi yerine getiren Apo A1 düzeylerinin ESLF'de daha düşük olduğunu göstermektedir. HDL-küçük alt fraksiyonlarındaki bu azalma, hepatik yetmezlikten kaynaklanan bozulmuş anabolizmaya bağlı olabilir.

Anahtar Kelimeler:

Son Dönem Karaciğer Yetmezliği, HDL Subfraksiyonları, Apo A1, CETP, LCAT

INTRODUCTION

End-stage liver failure, with distortion of hepatic architecture and formation of regenerative nodules, represents the late stage of progressive hepatic fibrosis (1). End-stage liver failure is an advanced stage of cirrhosis which is irreversible, and the only treatment option is liver transplantation. However, in some etiologic conditions (e.g. chronic viral hepatitis C and alcoholic liver disease) reversibility has been demonstrated in early-stage cirrhosis following treatment of the underlying cause (2).

Although varying geographically, alcoholism, chronic viral hepatitis C and non-alcoholic fatty liver disease (NAFLD) are the most common cause of ESLF, in western countries (3). Chronic viral hepatitis B is reported to be the main cause of ESLF in the Asia-Pacific region (4). Apart from these, there are many other less common causes such as hemochromatosis and Wilson's disease, primary biliary cirrhosis, primary sclerosing cholangitis and autoimmune hepatitis (5). Some cases with undetermined etiology are accepted as idiopathic or cryptogenic cirrhosis (5). Hepatic fibrosis, nodulation, and lobule destruction lead to decreased hepatocellular mass, decreased hepatic function and changes in blood flow in patients with ESLF (1).

Acting as a central organ in lipoprotein metabolism, the liver not only mediates selective cholesterol uptake from HDL but also facilitates HDL endocytosis and degradation (6). Cholesterol delivery to the liver, from peripheral tissues, is mediated by HDL (7). Apolipoprotein A1 (Apo A1), the major protein of HDL, is synthesized in the liver and enters the circulation where it interacts with various cell types through membrane transporters called ATP binding cassette transporter A1 (ABCA1) (8). This interaction allows the movement of cholesterol and phospholipids to Apo A1 resulting in the formation of mature HDL particles enriched in cholesterol. HDL interacts with scavenger receptor class B member 1 (SR-B1) receptors in the liver and transfers its cholesterol (9). Triglyceride can also be transported to circulating HDL from VLDL and low-density lipoprotein (LDL) particles by means of CETP activity. Liver Kupffer cells are known to be the main source of circulating CETP (10).

HDL is known to be protective against atherosclerosis and various studies have shown a negative correlation between plasma HDL-cholesterol (HDL-C) levels and the incidence of coronary artery disease (11, 12). As detailed above, the protective role of HDL against atherosclerosis stems from its involvement in a transport event called reverse cholesterol transport, which transports excess cholesterol from peripheral tissues back to the liver. Reverse cholesterol transport involves more than one step. These are respectively; transferal of free cholesterol from cells to HDL, esterification of free cholesterol in HDL by LCAT, and transfer of ester cholesterol to lipoproteins containing apolipoprotein (apo)-B via CETP (13). It has been suggested that disorders related to these steps involved in reverse cholesterol transport can be accountable for the occurrence of coronary artery disease. The question of whether cholesterol ester transfer from HDL to apo-B-containing lipoproteins via CETP is beneficial or harmful is still not fully clarified. There is research suggesting that CETP may have both an atherogenic and anti-atherogenic role (14). Due to its central function in reverse cholesterol transport, the LCAT enzyme is also an important protein and changes in LCAT protein levels have been implicated in the development of coronary artery disease (15).

Liver failure may cause dyslipidemia by impairing hepatic

lipid metabolism and in addition, impaired hepatic lipid metabolism may add to the advancement of chronic liver disease (16). Significant changes occur in both the level and the composition of lipoproteins and apolipoproteins in parallel to the severity of parenchymal damage in the liver (17). Lipid and lipoprotein changes that take place in ESLF can also be related to decreased levels and activity of circulating enzymes that modify lipoproteins such as LCAT and hepatic lipase (18). Impaired liver function significantly inhibits hepatic Apo A1 synthesis and Apo A1 serum levels are negatively correlated with severe liver damage (19). The hypothesis of our study was that ESLF could impact on HDL size and metabolism which could affect reverse cholesterol transport and endothelial protective properties of HDL. In this work, we targeted to compare HDL subfraction profile and HDL-related enzymes in healthy liver donors and in patients with liver failure due to varying etiology.

MATERIALS and METHODS

Study Groups

Blood samples were obtained from 20 voluntary liver donors and 20 liver failure patients. Nine liver failure patients had hepatic encephalopathy, while 11 did not. The age range of the study participants was between 19-65 years. The total of 40 subjects were divided into two groups as liver failure (n=20) and healthy donors (n=20). Subjects having oncological, hematological, respiratory system, central nervous system (CNS) disease, coronary insufficiency, renal failure, malnutrition, diabetes, traumatic brain injury or cadaver liver transplantation and who were receiving antipsychotic medication were excluded from the study. This study was reviewed and approved by the Akdeniz University Clinical Research Ethics Committee (08/02/2023 – Reference number: 70904504/67). The work was conducted in accordance with the principles of Research and Publication Ethics and the Helsinki Declaration; permission was obtained from relevant institutions to conduct the study, and informed consent was obtained from the participants, explaining the purpose and scope of the study. The Clinical Trials protocol record has been completed (ClinicalTrials.gov Identifier: NCT05958420).

Laboratory Measurements

Blood was collected from all patients before surgery. Triglycerides, total cholesterol and HDL-cholesterol were measured by enzymatic colorimetric methods on a Roche Cobas 8000 Modular Analyzer (Basel, Switzerland). LDL-cholesterol and VLDL-cholesterol were calculated by the Friedewald formula (20). Serum creatinine, albumin, ALT, AST, ALP and BUN were determined colorimetrically on a Roche Cobas 8000 Modular Analyzer.

HDL Subfraction Analysis

Quantimetrix Lipoprint HDL System (Redondo Beach, CA, USA) was used for the analysis of HDL subfractions as previously described (21).

Measurement of Apolipoprotein A1 Levels

Serum Apo A1 levels were determined with a human Apo A1 enzyme-linked immunoassay kit (catalog no. E1535Hu; Shanghai, China) following the company's guidelines. Apo A1 concentrations in the samples were calculated from a standard curve constructed with known Apo A1 standards.

Measurement of Cholesterol Ester Transfer Protein Levels

Serum CETP concentrations were measured with a human CETP enzyme-linked immunoassay kit (catalog no. E1515Hu; Shanghai, China) following the company's guidelines. CETP concentrations in the samples were calculated from a standard curve constructed with known CETP standards.

Measurement of Lecithin Cholesterol Acyltransferase Levels

Serum LCAT concentrations were measured with a human LCAT enzyme-linked immunoassay kit (catalog no. E2262Hu; Shanghai, China) following the company's guidelines. LCAT concentrations in the samples were calculated from a standard curve constructed with known LCAT standards.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software, version 9.0.0 for Windows operating system (GraphPad Software, San Diego, California, USA). A P value <0.05 was considered statistically significant. Statistical analysis for each measurement is indicated in the figure and table legends.

RESULTS

Serum Biochemistry

Laboratory values in donor and liver failure groups are given in Table I. Triglyceride, VLDL-cholesterol, ALT, AST, ALP, and BUN levels were significantly higher in the liver failure group compared to the donor group. Albumin levels were significantly lower in the liver failure group compared to the donor group. Total cholesterol, HDL-cholesterol, LDL-cholesterol, and creatinine levels were not significantly different between the groups.

Changes in HDL Subfractions

Figure 1A shows a representative liver failure group and donor gel tube after completion of electrophoresis. Electrophoretic migration takes place from the top to the bottom of the tube. LDL/VLDL is the slowest migrating band, while albumin is the most migrating particle. HDL particles are fractionated in the middle part of the gel. Figure 1B-C shows illustrations of densitometric scans of liver failure patient and donor groups. The distribution of HDL subfractions in donor and liver failure groups is given in Table II. HDL subfractions 1-3 and HDL-large concentrations were meaningly greater in the liver failure patients in

contrast to controls. HDL subfractions 5-10, HDL-intermediate and HDL-small concentrations were considerably reduced in the liver failure group in contrast to controls.

Apo A1 Levels

Apo A1 levels are shown in Figure 2A. Apo A1 levels were significantly lower in the liver failure group compared to the donor group. The Apo A1 levels (mean ± SEM) measured in the donor and liver failure groups were 3.98 ± 1.78 and 2.55 ± 0.92 g/L, respectively.

CETP Levels

CETP levels are shown as a bar graph in Figure 2B. Although not significant, a decrease in CETP activity was observed in the liver failure group compared to the donor group. The CETP levels (mean ± SEM) measured in the donor and liver failure groups were 2.20 ± 0.92 and 1.65 ± 0.22 µg/ml, respectively.

LCAT Levels

LCAT levels are shown in Figure 2C. No significant change was observed between the donor and liver failure groups. The LCAT levels (mean ± SEM) measured in the donor and liver failure groups were 4.37 ± 1.93 and 3.72 ± 0.30 µg/ml, respectively.

DISCUSSION

This study investigated HDL subfractions and HDL-related enzyme levels in patients with ESLF. The primary cause for liver failure in the patient group was cryptogenic cirrhosis (n=11), followed by hepatitis B (n=3), hepatitis C (n=2) and cancer (n=2). The etiology in the remaining patients was recorded as sclerosing cholangitis (n=1) and primary biliary cirrhosis (n=1). The Model for End-Stage Liver Disease (MELD) and the Child-Turcotte-Pugh scores (CTP) were used to assess prognosis in liver failure patients. The mean MELD and CTP scores calculated in liver failure patients were 16 and 7.7, respectively. A MELD score of > 40 and < 9 predicts a 71.3 % and 1.9 % mortality rate, respectively (22). The CTP score designates three classes as A = 5-6 points, B = 7-9 points and C = 10-15 points (18). Class A, B and C patients have 10, 30 and 70-80 % mortality rates, respectively (23). Serum bilirubin (mg/dL), serum creatinine (mg/dL) and International Normalized Ratio (INR) predict survival rate in MELD scoring (24) which is calculated by using the formula: 9.57 × loge (creatinine) + 3.78 × loge (total bilirubin) + 11.2 × loge (INR) + 6.43. CTP is graded by five measures of liver disease including ascites, encephalopathy, serum bilirubin (mg/dL), serum albumin (g/dL) and INR (23). Each measure is scored from 1 to 3, with 3 indicating the most severe dysregulation. The final CTP score is calculated by totaling the score for each parameter (23).

As previously mentioned, the major cause for ESLF in the patient group was cryptogenic cirrhosis, which leads to liver failure of unknown etiology without clear histologic and clinical criteria. Studies report that non-alcoholic steatohepatitis (NASH) is a predominant cause in the development of cryptogenic cirrhosis (25). The most common laboratory finding in NASH patients is a 2-3-fold increase in plasma serum transaminase levels and ALP levels may also be elevated in patients (26). A significant decrease in serum albumin, hypertriglyceridemia and elevated VLDL are also reported in cases with NASH, as observed herein (26, 27) (Table I).

Table I. Laboratory values in donor and liver failure groups

Variable	Donor (n=20)	Liver failure (n=20)
Triglyceride (mg/dL)	104.1± 34.05	136.6 ± 43.28*
Total Cholesterol (mg/dL)	179.90 ± 39.99	203.80 ± 42.61
HDL-Cholesterol (mg/dL)	50.55 ± 16.75	45.52 ± 17.70
LDL- Cholesterol (mg/dL)	110.30 ± 25.97	95.50 ± 24.81
VLDL-Cholesterol (mg/dL)	17.27 ± 5.65	22.67 ± 7.19*
Creatinine (mg/dL)	0.69 ± 0.23	0.74 ± 0.17
ALT (U/L)	23.65 ± 18.29	60.54 ± 46.07*
AST (U/L)	20.95 ± 5.74	75.40 ± 43.80*
ALP (U/L)	75.35 ± 21.74	187.30 ± 151.80*
BUN (mg/dL)	11.48 ± 3.02	16.37 ± 7.90*
Albumin (g/dL)	4.27 ± 0.35	2.97 ± 0.67*

All values are mean ± SD. Statistical analysis was performed by Student's t test or Mann Whitney U test.*p<0,05 vs. donor group. Abbreviations: HDL, high-density lipoprotein; LDL low-density lipoprotein; VLDL, very low-density lipoprotein; ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; BUN, blood urea nitrogen.

NASH is different from steatosis in terms of its course. It can progress to fibrosis, leading to cirrhosis and even death from terminal liver disease. In general, fibrosis develops in half of patients with NASH, while 15% develop cirrhosis and 3% develop liver failure or progress to liver transplantation (28).

Specific enzyme deficiencies are held responsible for lipid and lipoprotein changes observed in liver failure. The initial phase of HDL synthesis occurring in the liver includes the production of Apo A1. Apo A1 is the operational protein of HDL and initiates the development of pre-beta HDL by taking up cholesterol and phospholipids from hepatocytes via the ABCA1 transporter (29). Circulating HDLs are lipoproteins in the density range 1.063-1.21 g/ml. The small Apo A1-containing HDL particles (pre-beta HDL) are characterized by pre-beta mobility on agarose gel electrophoresis, while most HDL particles migrate with alpha mobility (30). HDL lipoproteins are also separated by non-denaturing polyacrylamide gradient gel electrophoresis. Subfractions 1-3 match up to large HDL, while 4-7 include intermediate HDL subfractions. Subfractions 8-10 are classified as small HDL particles. HDL subfraction analysis in our study showed a clear change towards the larger HDL subclasses in liver failure patients and a significant reduction in smaller HDL subfractions (Figure 1 and Table II).

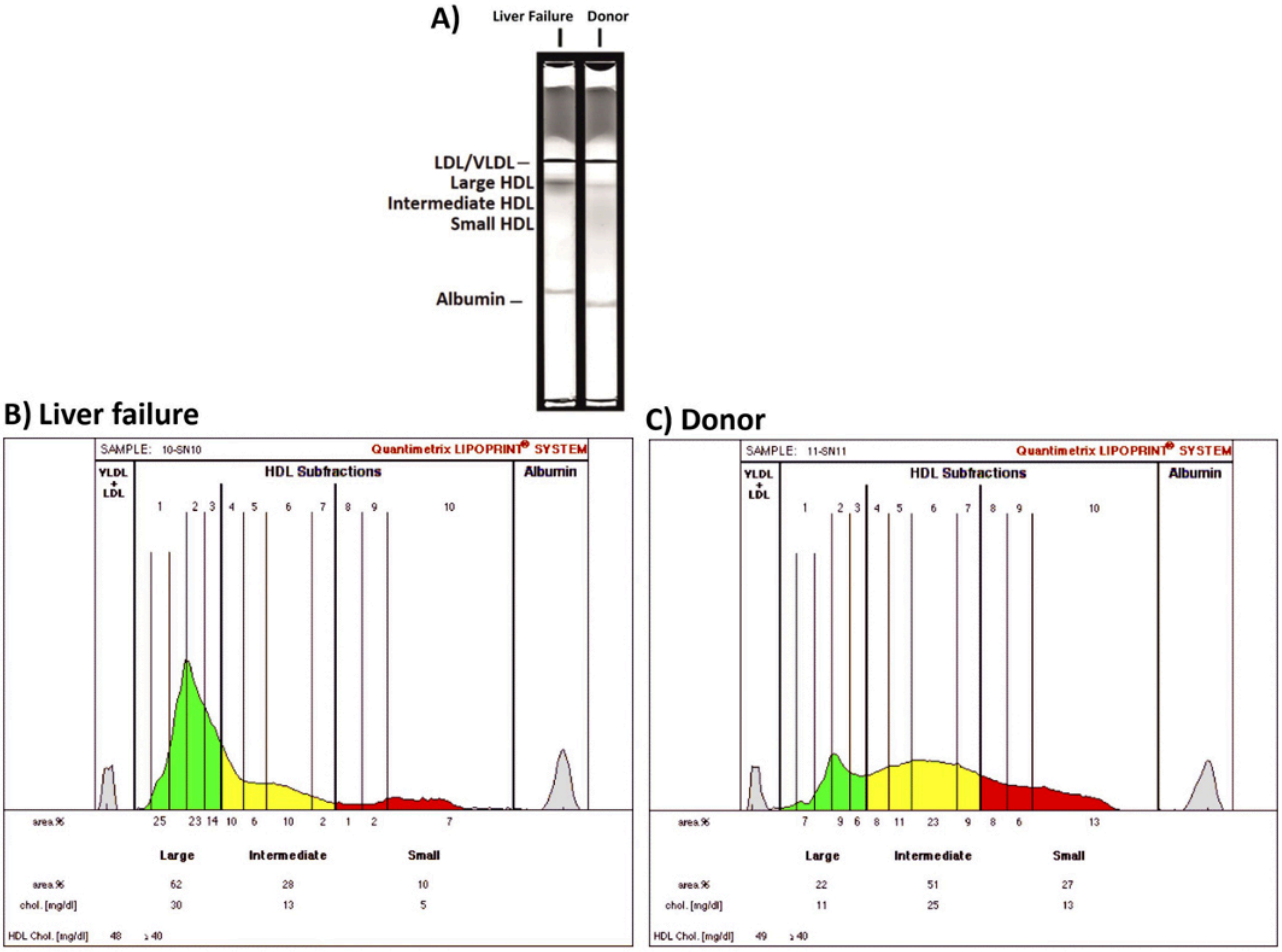

Figure 1. Electrophoresis of High-Density lipoproteins A) Appearance of electrophoretic relocation of two gel tubes from a subject with liver failure and a healthy donor. B) Illustrative densitometric scan of a liver failure subject. C) Illustrative densitometric scan of a donor.

Table II. HDL subfraction analysis in donor and liver failure groups

Variable	Donor (n=20)	Liver failure (n=20)
HDL-1 (%)	10.46 ± 3.73	26.62 ± 10.06*
HDL-2 (%)	10.72 ± 3.99	18.93 ± 5.48*
HDL-3 (%)	7.45 ± 3.58	10.10 ± 2.88*
HDL-4 (%)	8.96 ± 3.36	8.73 ± 1.29
HDL-5 (%)	9.04 ± 1.79	6.73 ± 2.34*
HDL-6 (%)	18.90 ± 3.70	11.15 ± 5.21*
HDL-7 (%)	8.15 ± 2.42	3.48 ± 2.16*
HDL-8 (%)	7.26 ± 2.22	2.89 ± 1.77*
HDL-9 (%)	6.09 ± 1.85	2.57 ± 1.31*
HDL-10 (%)	12.99 ± 4.80	8.22 ± 3.53*
HDL Large (%)	28.63 ± 9.49	55.64 ± 13.87*
HDL Inter. (%)	45.06 ± 4.23	30.10 ± 10.01*
HDL Small (%)	26.34 ±8.20	13.69 ± 5.88*

All values are Mean ± SD. Statistical analysis was performed by Student's t-test or Mann Whitney U test. HDL; high-density lipoprotein, Inter.; intermediate. *, p<0.05 vs. donor group.

The significant decrease in the smaller HDL fractions observed in liver failure is likely due to impaired synthesis of Apo A1 by the liver. Indeed, there was a noteworthy drop in circulating Apo AI concentrations in liver failure subjects (Figure 2A). There are studies reporting that decreases in plasma apolipoprotein levels are positively correlated with the severity of liver failure (31).

We observed a nonsignificant reduction in LCAT activity in liver failure patients (Figure 2C). Studies have reported that LCAT activity is correlated with plasma albumin values which was significantly decreased in the liver failure group (32). Depression of LCAT activity in liver failure leads to an increase in free cholesterol and lecithin levels with a parallel decrease in cholesterol ester and lysolecithin content (33). Studies have reported that reduced LCAT activity in liver failure leads to low cholesterol ester levels in HDL lipoprotein which are comparably enriched in phospholipids and triglycerides (34). No major changes were observed in CETP levels among the donor and liver failure groups (Figure 2B). Our results are in accord with earlier reports that have also shown normal levels of CETP in liver cirrhosis (18).

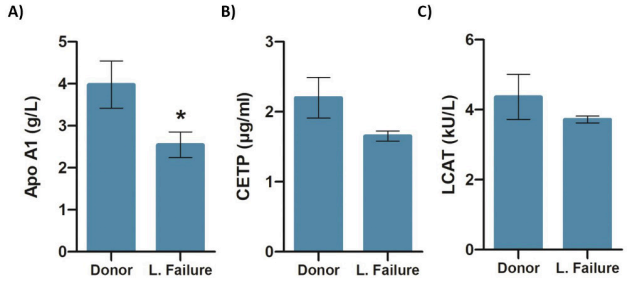

Figure 2. A) Serum apolipoprotein A1 (Apo A1) protein levels. Values are mean ± SEM (n=20). Statistical analysis was done by Student's t test. *, p<0,05 vs. donor group. B) Serum CETP levels. Values are mean ± SEM (n=20). Statistical analysis within each group was done by Mann-Whitney U test. No significant difference was observed among the groups. C) Serum LCAT levels. Values are mean ± SEM (n=20). Statistical analysis within each group was done by Mann-Whitney U test. No significant difference was observed among the groups.

Studies evaluating the risk of coronary artery disease in ESLF are conflicting. The etiology of liver failure is important in the development of coronary artery disease. Despite studies showing that the risk of atherosclerosis is high in NASH-dependent liver failure there are also studies reporting that liver failure is protective against coronary artery disease (35, 36). Studies have shown that HDL subfraction analyses are more predictive of coronary artery disease risk than total HDL cholesterol measurements (37). HDL-large shows an inverse correlation with atherosclerosis formation while HDL-small shows a direct correlation (38). We observed that HDL-large concentrations were notably higher, while HDL-small concentrations were considerably reduced in the liver failure subjects in contrast to the donors, suggesting a decreased risk for atherosclerosis in the liver failure group. On the other hand, liver failure patients have decreased Apo A1 levels which may well unfavorably influence the protective function of HDL against inflammation and oxidative stress (39).

Limitations

It is worth noting some limitations of the work. CETP and LCAT concentrations were only determined by ELISA. However, we can't rule out that both levels and activities of these enzymes can contribute to the HDL changes observed in ESLF. Additional studies are also needed to determine LDL subfraction profile in ESLF patients and its possible role in the assessment of atherosclerosis risk in ESLF.

CONCLUSION

In summary, our work reveals that liver failure significantly alters HDL subfractions and Apo A1 levels. Accordingly, such changes in HDL composition may be clinically pertinent and expand our capability to screen the progress of liver failure in associated patients.

Ethics Committee Approval:
This research complies with all the relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration, and has been approved by the Akdeniz University Clinical Research Ethics Committee (08/02/2023 – Reference number: 70904504/67).

Informed Consent:
All the participants' rights were protected and written informed consents were obtained before the procedures according to the Helsinki Declaration.

Author contributions:
Concept - Z.B., M.A.; Design - Z.B., M.A.; Supervision - Z.B., M.A.; Resources - M.A.; Materials - M.A., B.D., İ.A.; Data Collection and/or Processing – M.A., B.D., İ.A., T.Ç., Ç.Y., A.Ö., B.Ç.; Analysis and/ or Interpretation - T.Ç., Ç.Y., A.Ö., B.Ç.; Literature Search - M.A., T.Ç.; Writing Manuscript - M.A., Z.B.; Critical Review – Z.B.

Conflict of Interest:
The authors have no conflict of interest to declare.

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Data availability
Data obtained and analyzed in the work are not publicly available due to ethical limitations involving human subjects but are available from the corresponding author on reasonable request.

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