Cirrhosis is a common disease with high death and morbidity rate around the world.¹ Cirrhosis is caused by chronic liver inflammation, which proceeds to diffuse hepatic fibrosis. End-stage liver failure occurs when the normal hepatic structure is replaced by regenerating hepatic nodules.² Chronic liver inflammation does not usually result in cirrhosis; but, if it does, the time it takes to develop cirrhosis can range from weeks in individuals with severe biliary blockage to decades in persons with chronic etiologies such as viral hepatitis. Cirrhosis can have an asymptomatic (initial) phase followed by a symptomatic phase that might last months to years.

In the absence of a liver transplant, this symptomatic phase, known as decompensated cirrhosis, is associated with repeated hospitalizations, a decrease in patients’ quality of life, and even mortality.³,⁴ Cirrhosis was responsible for 2.4% of global deaths in 2019.⁵ Cirrhosis is mostly caused by HCV infection, alcoholic liver disease, HBV infection, and non-alcoholic fatty liver disease (NAFLD).⁶ In other words,
etiologies can be classified into viral (HBV, HCV, HDV), alcohol-related (alcoholic liver disease), autoimmune (primary biliary cholangitis, autoimmune hepatitis, primary sclerosing cholangitis), biliary (biliary strictures, biliary atresia), vascular (veno-occlusive disease, Budd-Chiari syndrome etc.), metabolic and genetic (NAFLD, type IV glycogen storage disease, α1-antitrypsin deficiency, Wilson’s disease, cystic fibrosis, lysosomal acid lipase deficiency, hemochromatosis, progressive familial intrahepatic cholestasis, tyrosinemia type 1), chronic drug-induced and unknown (cryptogenic) causes. Cryptogenic cirrhosis is a case of hepatic cirrhosis in which the exact etiology cannot be determined despite detailed clinical, laboratory, and histological evaluations. In fact, a diagnosis of exclusion is made to perform the diagnosis of cryptogenic cirrhosis and the diagnosis is made when the etiologies causing cirrhosis are ruled out. The incidence of cryptogenic cirrhosis decreases considerably by the development of new diagnostic tools and defining the factors causing cirrhosis. Although it has been said that the prevalence of cryptogenic cirrhosis is 5%-30% in previous studies, there are no definite results since most of these results were obtained from studies conducted in single centers. When transplant databases are examined, it is reported that the prevalence of cryptogenic cirrhosis is around 10%.

The cause and severity of liver disease have changed dramatically during the last decade. The epidemiology and severity of cirrhosis are changing due to the increasing prevalence of obesity and alcohol consumption and due to advances in the management of HBV and HCV virus infections. For this reason, new markers and diagnostic criteria are needed to determine cirrhotic patients and also to discriminate among the different etiologies and stages of cirrhosis.

An enzyme called lysosomal acid lipase (LAL) controls the intra-lysosomal hydrolysis of triglycerides and cholesterol esters to create free cholesterol and fatty acids. A mutation in the LIPA gene results in the uncommon autosomal recessive genetic condition known as LAL deficiency, which is characterized by triglyceride and cholesterol ester buildup in a number of organs. In hepatocytes and macrophages (Kupffer cells), LAL deficiency causes a buildup of TG and cholesterol esters, which encourages chronic inflammation and fibrosis. Given the high incidence of severe liver fibrosis in the LAL deficient condition and the rapid development of cirrhosis, it is likely that the accumulation of lysosomal triglycerides and cholesterol ester is one of the main causes of liver fibrosis. According to a recent study, mice with hepatocyte-specific LAL deletion had increased transaminases as well as elevated levels of hepatic cytokines and chemokines, which led to Kupffer cell activation and liver damage.

In the present study, it is aimed to evaluate LAL activity in patients with cirrhosis. Also, our secondary aim was aimed to compare LAL activity with cirrhosis patients of known etiology to check whether LAL activity can aid determine patients with cryptogenic cirrhosis by using machine learning methods.

Methods

Dataset and Patient Characteristics

An open-access data set was utilized in the study. In the data set used, the patients consist of three groups. There were 63 patients diagnosed with cryptogenic cirrhosis in the first group (cryptogenic group), 88 patients with cirrhosis of known etiology in the second group (non-cryptogenic group), and 97 healthy individuals without clinically important liver disease in the third group (control group). The patient groups in the study were matched in terms of gender and age.

Modelling Phase

Stochastic Gradient Boosting (SGB), a tree-based technique from machine learning methods, was utilized in the modelling phase to model the control group and patients with cirrhosis and to explore the effect of LAL. Friedman invented stochastic gradient boosting by integrating randomization into the gradient boosting approach. A random subsample is chosen at each refresh in SGB using a permutation sampling approach. This subsample, rather than all learners, is used to generate the model update, lowering the correlation between trees. This method, like other ensemble learning methods, does not produce massive trees; instead, each tree (approximately 100-200 trees) generated during the process is summed up, and each observation is classified based on the most common categorization across trees. These distinctions distinguish the SGB approach from other augmentation techniques and reduce its unbalanced data sets and sensitivity to outliers. This approach, which has a very high predictive power compared to other algorithms, is 5 times faster than other methods and incorporates a series of regularizations that can improve the overall performance of the model while decreasing overfitting and overlearning. The data are separated as 80% training 20% test data. To confirm model validity, the n-fold cross-validation method, one of the resampling methods, was used in this work. In this method; The dataset is first separated into n pieces, after which the model is applied to those pieces. In the second step, one of
the n parts is used for testing, while the remaining n-1 parts are used for training. In the last stage, the cross-validation approach is evaluated using the average of the values collected from the models. Accuracy (Acc), balanced accuracy (bacc), sensitivity (se), specificity (sp), positive predictive value (ppv), negative predictive value (npv), and F1-score measures were utilized to assess the modeling performance. The graphical summary showing the method, analysis and modeling process applied in the study is shown in Figure 1.

Biostatistical Analysis
In the study, data were summarized as median (95 percent confidence intervals), and number (percentage). The Kolmogorov-Smirnov test was used to determine whether or not the data was normal. The Mann-Whitney U test was utilized for statistical analysis of non-normally distributed data. p<0.05 was regarded statistically significant. Analyzes were performed with IBM SPSS Statistics 25. Since the open access data set was used in this study, ethical approval was not required.

Results
The mean age of all patients and healthy individuals utilized in the study was 66.24±9.51. When the mean age of the groups is examined, the mean age of the cryptogenic patients was 68±10, the mean age of the cirrhosis patients with known etiology was 65±10 years, and the mean age of the control group was 66±8 years.

Comparison of Control and Cryptogenic Cirrhosis Patients
When the total cholesterol, triglycerides, LDL cholesterol, AST, ALT, HDL cholesterol, platelets, white blood cells, and LAL activity variables were examined for the control and cryptogenic groups, a significant difference was found between the two groups in all variables except triglycerides. Table 1 shows the findings of the analysis.

Comparison of Control and Cirrhosis of Known Etiology
When the total cholesterol, triglycerides, LDL cholesterol, AST, ALT, HDL cholesterol, platelets, white blood cells, and LAL activity variables were examined for the control and non-cryptogenic groups, significant differences were obtained between the 2 groups in all variables. Table 2 shows the findings of the analysis.

Comparison of Cryptogenic Cirrhosis and Cirrhosis of Known Etiology
When the total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, AST, ALT, platelets, white blood cells, LAL activity and spleen volume variables were examined

<p>| Table 1. Comparison of control and cryptogenic cirrhosis groups in terms of variables |
|---------------------------------|----------------|----------------|-----------|</p>
<table>
<thead>
<tr>
<th>Variables [Median (95 % CI)]</th>
<th>Control group</th>
<th>Cryptogenic group</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>200 (194-213)</td>
<td>145 (128-152)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL Cholesterol (mg/dL)</td>
<td>123 (113-128)</td>
<td>72 (66-83)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dL)</td>
<td>50 (47-53)</td>
<td>36 (35-40)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>116.5 (108-125)</td>
<td>131 (115-159)</td>
<td>0.053</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>17 (16-20)</td>
<td>39 (33-42)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>24.5 (23-27)</td>
<td>38 (34-45)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Platelets (cells/mm³)</td>
<td>225 (215-244)</td>
<td>105 (96-125)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WBC (cells/mm³)</td>
<td>6.02 (5.8-6.54)</td>
<td>4.89 (4.36-5.35)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LAL activity (nmol/Spot/H)</td>
<td>0.96 (0.89-1.08)</td>
<td>0.62 (0.52-0.76)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* : Mann Whitney U test.
for the cryptogenic and non-cryptogenic groups, only total cholesterol, HDL and triglyceride variables were statistically different. Table 3 shows the findings of the analysis.

**Modelling Results**

The results of the performance measurements obtained from the modeling using cirrhotic patients and control individuals in the test and training set are provided in Table 4. The graph of the significance of the variables obtained from the modeling is given in Figure 2. When the significance of the obtained variables is examined, it is seen that the most important variables that explain the dependent variable (control/cirrhosis) are platelet, LAL activity, ALT, AST, and white blood cells, respectively.

**Discussion**

Chronic liver disease (CLD) and cirrhosis cause a high burden of disability and increased healthcare costs, in addition to 2 million deaths worldwide each year. An accurate estimation of the burden of cirrhosis is vital for setting research and policy priorities. For this reason, worldwide studies have gained importance to provide early diagnosis and treatment of cirrhosis. There is a need for new markers that can detect cirrhosis at early stages. Therefore, the current study focused on the changes in the LAL activity in patients with cirrhosis. Within the scope of the study, in addition to the LAL activity variable, total cholesterol, AST, ALT, LDL cholesterol, HDL cholesterol, platelets, triglycerides, and white blood cells variables were examined in cryptogenic cirrhosis, cirrhosis with a known etiology and control groups, and their changes between groups were determined. In addition, to determine the importance of the LAL activity in the cirrhosis and control groups, modeling was performed using the SGB method which is one of the machine learning technique.

When the results were examined, significant statistical differences were obtained in all the variables examined (except for the triglycerides variable) in the analyses performed in healthy control and cryptogenic cirrhosis patients. Variables that differ were total cholesterol, platelets, LDL cholesterol, AST, HDL cholesterol, ALT, white blood cells, and LAL activity.

**Table 2.** Comparison of control and non-cryptogenic cirrhosis groups in terms of variables

<table>
<thead>
<tr>
<th>Variables [Median (95 % CI)]</th>
<th>Control group</th>
<th>Non-cryptogenic group</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>200 (194-213)</td>
<td>148 (135-166)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL Cholesterol (mg/dL)</td>
<td>123 (113-128)</td>
<td>85 (73-92)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dL)</td>
<td>50 (47-53)</td>
<td>45 (39-49)</td>
<td>0.012</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>116.5 (108-125)</td>
<td>94 (85-102)</td>
<td>0.012</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>17 (16-20)</td>
<td>31 (27-46)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>24.5 (23-27)</td>
<td>29 (26-34)</td>
<td>0.036</td>
</tr>
<tr>
<td>Platelets (cells/mm³)</td>
<td>225 (215-244)</td>
<td>114 (92-125)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WBC (cells/mm³)</td>
<td>6.02 (5.8-6.54)</td>
<td>5.34 (4.77-6.03)</td>
<td>0.002</td>
</tr>
<tr>
<td>LAL activity (nmol/Spot/H)</td>
<td>0.96 (0.89-1.08)</td>
<td>0.54 (0.48-0.67)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*: Mann Whitney U test.

**Table 3.** Comparison of cryptogenic and non-cryptogenic cirrhosis groups in terms of variables

<table>
<thead>
<tr>
<th>Variables [Median (95 % CI)]</th>
<th>Cryptogenic group</th>
<th>Non-cryptogenic group</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>145 (128-152)</td>
<td>147.5 (135-166)</td>
<td>0.139</td>
</tr>
<tr>
<td>LDL Cholesterol (mg/dL)</td>
<td>72 (6-83)</td>
<td>85 (73-92)</td>
<td>0.037</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dL)</td>
<td>36 (35-40)</td>
<td>45 (39-49)</td>
<td>0.023**</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>132.5 (115-159)</td>
<td>94 (85-102)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>39 (33-42)</td>
<td>31 (27-46)</td>
<td>0.681</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>37.5 (32-44)</td>
<td>29 (26-34)</td>
<td>0.059</td>
</tr>
<tr>
<td>Platelets (cells/mm³)</td>
<td>105 (96-130)</td>
<td>114 (92-125)</td>
<td>0.986</td>
</tr>
<tr>
<td>WBC (cells/mm³)</td>
<td>4.93 (4.36-5.4)</td>
<td>5.34 (4.77-6.03)</td>
<td>0.095</td>
</tr>
<tr>
<td>LAL activity (nmol/Spot/H)</td>
<td>0.62 (0.52-0.76)</td>
<td>0.54 (0.48-0.67)</td>
<td>0.450</td>
</tr>
<tr>
<td>Spleen volume (cm³)</td>
<td>70 (59-80)</td>
<td>59 (51-67)</td>
<td>0.151</td>
</tr>
</tbody>
</table>

*: Mann Whitney U test.
The decrease of 0.34 units in LAL activity in the cryptogenic cirrhosis group is statistically significant compared to the control group. The LAL activity value is lower in cryptogenic cirrhosis patients than in the control group. In the analysis performed in control and cirrhosis with known etiology, significant statistical differences were obtained in all variables including total cholesterol, AST, LDL cholesterol, triglycerides, HDL cholesterol, ALT, platelets, white blood cells, LAL activity. The decrease of 0.42 units in LAL activity in cirrhosis with known etiology is statistically significant compared to the control group. The results of our study show that the LAL activity value is at a lower level in cirrhosis patients with known etiology compared to the control group. If the results obtained for the cryptogenic and known etiology cirrhosis group are compared, it was observed that significant differences are obtained in total cholesterol, HDL, and triglycerides variables. It was observed that the LAL activity did not differ significantly between both groups (cryptogenic and non-cryptogenic groups) with cirrhosis.

The LAL activity decreased in both cirrhosis patient groups (cryptogenic and non-cryptogenic) compared to the control group, while there is no differential effect between the cryptogenic and non-cryptogenic cirrhosis groups.

When the modeling results were conducted to determine the importance of LAL activity in the control and cirrhosis patient groups; accuracy (ACC), balanced accuracy (BACC), Sensitivity (SE), Specificity (SP), positive predictive value (PPV) and negative predictive value (NPV), and F1 score were 93.2%, 93.4%, 94.4%, 92.3%, 89.5%, 96%, and 91.9%, respectively. According to the performance metric values obtained from here, we can assume that the control and cirrhosis patient group can be classified with high accuracy by modeling with the independent variables (total cholesterol, triglycerides, LDL cholesterol, platelets, AST, HDL cholesterol, ALT, white blood cells, and LAL activity). When the variable importance values produced from the modeling are reviewed, platelets are found to be the most important variable related to cirrhosis. In the second row, in accordance with the purpose of the study, we see that the LAL activity variable is among the most influential variables on the dependent group. The LAL activity variable is followed by ALT, AST, white blood cells, HDL cholesterol, LDL cholesterol, triglycerides, and total cholesterol variables, respectively.

Based on the results obtained in the cirrhosis patient group compared to the control group, when the studies conducted with the platelet variable with the highest variable importance value were examined, it was determined that the platelet value was lower in patients with cirrhosis compared to the control group.\(^{[22, 23]}\) Similarly, in the current study, the platelet value was found to be lower in both cryptogenic and cirrhosis groups with known etiology compared to the control. However, no difference in platelet value was observed between cryptogenic and known etiology patients with cirrhosis. The decrease in LAL activity in the cirrhosis group compared to the control is striking. However, there is no significant difference in LAL activity between cryptogenic and cirrhosis groups with known etiology. It was reported that the LAL activity was low in the cryptogenic patients.\(^{[24]}\) Another study showed reduced LAL activity in patients with cirrhosis.\(^{[18]}\) In the current study, it was determined that LAL activity that is an important variable in cirrhosis and it was validated by machine learning methods in addition to basic statistical tests. Other predictive variables include AST and ALT, and the values of these markers increase in liver disease.

In conclusion, the LAL activity can be considered as a valuable parameter that can be examined in cirrhosis patients. Currently large sample size studies are required to evaluate the value of LAL activity to discriminate among the different etiologies of cirrhosis.

**Disclosures**

**Ethics Committee Approval:** This article was produced from open-access dataset (https://doi.org/10.1371/journal.pone.0156113.s001). Therefore, it has been reported by the institute that ethics committee approval is not required.


