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ORIGINAL RESEARCH

Serum Kallistatin - A Novel Biomarker for Alcoholic Liver Disease

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ABSTRACT

Background:Kallistatin is a glycoprotein found in human plasma. Kallistatin is mostly generated by the liver, with minor quantities made by the kidney, pancreas, heart, lung, and colon. In the tissue kallikrein – kinin system, kallistatin acts as a balancing agent. Kinins, kininogens, and kallikrein, kallistatin, and bradykinin receptors are all part of this system. When compensated disease sets in, kallistatin can be employed as a critical biomarker in detecting alcoholic liver disease early. In the early stages of liver disease, it is normally quiet and asymptomatic, but as it progresses to the symptomatic decompensated stage, it can cause serious consequences. The patient's prognosis is dismal, with portosystemic encephalopathy, variceael haemorrhage, and hepatocellular cancer. The current study was done to evaluate the use of Kallistatin as a non-invasive marker in the diagnosis of alcoholic liver disease (ALD), and to compare serum Kallistatin levels in alcoholic liver disease patients and healthy controls.

Materials and Methods: This study includes sixty patients with alcoholic liver disease (divided into two groups based on compensated and decompensated features) and thirty healthy controls. Total and direct bilirubin, AST, ALT, ALP, GGT, albumin, and serum Kallistatin concentrations were determined using a regular automated analyzer and Kallistatin levels were analyzed using an Enzyme Linked Immunosorbent Assay. The t-test, Pearson Spearman rank correlation, Anova, and Receiver operating characteristic (ROC) Curve have been used to examine the data (SPSS version 16.0 software).

Results: ThemeanserumKallistatin levelswere(25.22 ±3.62µg/mL),(15.2 ±3.8 µg/mL)and(13± 3.3µg/mL) in Control group,compensatedgroup and decompensatedgroup .A statistically significant p value (p value 0.001) was obtained. As liver damage progresses, Kallistatin levels decrease. In alcoholic liver disease patients, serum levels of AST, ALT, ALP, and GGT were increased, while albumin levels decreased, which was statistically significant. SerumKallistatin and albumin levels have a significant positive correlation (p= 0.05), while Serum Kallistatin and GGT levels have a significant negative correlation (p= 0.05). For Kallistatin , ANOVA revealed a statistically significant p value of 0.001 and a ROC with optimal cut off value of 0.922.

Conclusion: SerumKallistatin levelscanplay avitalandprotectivevariableinpreventingalcoholicliverdisease. This study leads a pathway for therapeuticinterventionto be started earlieron the basisofserumKalli statinlevels.Estimationofserum Kallistatin levelscanbeaddedinroutineinvestigationsforliverfunctiontestsin patientswithalcoholicliverdisease.

Keywords: Serum Kallistatin, Alcoholic Liverdisease,Biomarker,Alcoholism.

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INTRODUCTION

Alcohol-associated liver disease (ALD) and liver-related deaths, which are caused by acute or chronic alcohol consumption, are among global health-care costs. Chronic alcohol intake damages the liver's regular defence mechanisms and is likely to interrupt the gut barrier system, particularly mucosal immune cells, leading to reduced food absorption. The management for alcohol-associated liver disease is dictated by the type of liver injury that culminates in fatty liver, hepatitis, and cirrhosis. Chronic alcohol consumption, obesity, hereditary factors, and viral hepatitis are all risk factors for liver damage. The quantity, duration, and pattern of alcohol intake all lead to liver failure.[1,2]

However, if alcohol is consumed consistently, alcohol-associated liver disease will proceed to alcoholic hepatitis, an inflammatory condition that destroys liver cells. Continued alcohol usage wreaks havoc on the liver, leading to the ultimate stage of alcohol-associated liver disease.[3,4] Cirrhosis of the liver, also known as end-stage alcohol-associated liver disease , is characterised by irreversible scarring, a decline in liver function, and eventually liver failure and death. The following are the three stages of liver disease.[5,6]

Fatty Liver: A accumulation of fat in the liver is the cause of alcoholic fatty liver disease. Fatty liver (or hepatic steatosis) that is very mild might be asymptomatic and develop even with modest alcohol use, however frequent drinking increases the risk. With continued abstinence from alcohol, this condition can be prevented or reversed.

Alcoholic hepatitis: Continued drinking can lead to chronic liver inflammation, known as alcoholic hepatitis. Symptomatic hepatitis may include some jaundice and an enlarged liver. More advanced disease may include anemia and coagulation problems. Attempts to manage alcoholic hepatitis may include alcohol abstinence, nutritional support, and in some cases, corticosteroid therapy. If drinking continues, the condition may become progressively severe, with chronic inflammation eventually leading to irreversible necrosis of liver tissue.

Cirrhosis of the liver: Cirrhosis of the liver occurs when hepatic fibrosis and scar tissue become extensive, potentially resulting in portal hypertension and a shrunken, non-functioning liver. Cirrhosis of the liver is irreversible. However, quitting alcohol can stop it from getting worse.

However,ifproblemsduetoalcoholintake are diagnosed earlier, a physician can easilypreventfurtherprogression toalcoholicliverdisease.Recently development of few biomarkershelped in early detection of alcohol induced liver disease , and early intervention to counsel the patient for abstinence fromalcohol and also help to monitor progression while patient is ontreatment.

As a new serine proteinase inhibitor (Serpin) and a particular tissue kallikrein inhibitor, kallistatin was initially isolated and described from human plasma. Kallistatin possesses anti-angiogenic, anti-inflammatory, anti-tumor, anti-oxidant, and anti-tumor properties. Many human tissues, including as the eye, kidney, liver, heart, arteries and veins, blood cells, and bodily fluids, contain it. Several investigations have found that the liver is the primary location of Kallistatin production and secretion, suggesting that it might be used as a biomarker for liver cirrhosis.[7,8]

The serpinA4 gene encodes kallistatin (Kallikrein-Binding Protein – KBP, Serpin A4), an acidic glycoprotein of 427 amino acids and a molecular weight of 58 kDa. It is a selective endogenous inhibitor of tissue kallikrein and belongs to the serine proteinase inhibitor (SERPIN) class. Its primary physiological function is to prevent inflammation, fibrosis, and

oxidative damage. Kallistatin lowers collagen fraction volume, as well as collagen type I and II deposition and expression. Furthermore, it inhibits fibronectin and collagen expression in cultured mesangial cells by decreasing the production of angiotensin II–induced reactive oxygen species and transforming growth factor-1 expression. Kallistatin inhibits tumour development and angiogenesis by decreasing endothelial cell proliferation, migration, and adhesion in response to VEGF and bFGF.[9,10]

Tissue kallikreins are a family of serine proteinases well recognised for their capacity to break down plasma kininogens into kinins that produce vasodilation. It belongs to the serpin family and is a medium-sized protein. Although the term SERPIN stands for Serine Protease Inhibitor, it is a misnomer. Serpin family proteins are not all serine protease inhibitors, and vice versa. It's an acute phase reactant with a negative charge. During sepsis, kallistatin forms a covalent binding complex with tissue kallikrein and declines.[11-13]

There are two structural parts in the Kallistatin protein: an active site and a heparin-binding domain. The active site is required for the development of a complex with tissue kallikrein and, as a result, tissue kallikrein inhibition. Kallistatin's inhibitory function is inhibited when it binds to heparin.

The heparin binding domain, on the other hand, is required for antagonising VEGF, tumour necrosis factor (TNF)-, high mobility group box-1 (HMGB1), and transforming growth factor (TGF)-mediated signalling pathways. A vast range of human organs, including the kidney, liver, eye, heart, prostate, colon, blood arteries, and blood cells, manufacture and release kallistatin.

Kallistatin administration via gene or protein delivery has been shown to protect animals from a variety of pathological conditions, including hypertension, cardiovascular and organ damage, arthritis, sepsis, influenza virus infection, tumour growth and metastasis, and has the potential to be used as a therapeutic agent or target in animal models.[14,15]

In individuals with liver illness, obesity, septic syndrome, severe pneumonia, inflammatory bowel disease, and colon and prostate cancer, kallistatin levels in the circulation, bodily fluids, and tissues are much decreased.Although Kallistatin levels in the vitreous fluid of diabetic retinopathy patients were found to be lower, circulating Kallistatin serum levels were found to be higher in type 1 diabetes patients with vascular problems. Similar findings were found in type 2 diabetes patients, who had a rise in serum Kallistatin, which corresponds with the occurrence of retinopathy. According to a recent study, blood Kallistatin levels are considerably lower in patients with liver cirrhosis (LC) but not in individuals with hepatocellular carcinoma, and this decline is linked to the severity of LC and disruption of normal liver function.

Kallistatin is a plasma protein that binds to tissue kallikrein and diminishes as liver damage from drinking advances. Kallistatin is a protein that protects cells against apoptosis. Anti-angiogenic, anti-tumor, and anti-oxidant activities are all present. As a result, the goal of this study was to see if Kallistatin might be used as a non-invasive marker for detecting and evaluating the severity of alcoholic liver damage.

Aim& Objectives:

To evaluate the role of Kallistatin as a non-invasive marker in the diagnosis of alcoholic liverdisease.ToassessthediseaseseveritywithserumKallistatin levels.To compare serum Kallistatin levels in alcoholicliverdiseasepatientswithapparentlyhealthyindividuals.

MATERIALS & METHODS

The Department of Biochemistry and the Department of Medicine, Surabhi Institute of Medical Sciences, Siddipet, collaborated on this case control research from July 2020 to November 2021.Serum Kallistatin levels are measured in control groups and cases are

divided into two groups: compensated (or asymptomatic) and decompensated (or progressing) groups to achieve the study's goal. AST, ALT, ALP, GGT, total and direct bilirubin, and albumin are among the other markers tested.

Studypopulation: A total of 90 human individuals participated in our investigation. During their outpatient visit for non-hepatic reasons, controls were chosen from the outpatient department. We chose 30 people who appeared to be in good health, had a history of complete abstinence from alcohol, and had a normal ultrasonography abdomen. 60 patients were chosen from the Surabhi Institute Of Medical Sciences in Siddipet's General Medicine department. After receiving ethics committee permission, the research was carried out. All of the participants in the research gave their consent. Patients with alcoholic liver disease (CASES). Compensated and decompensated traits were used to divide the patients into two groups. 30 individuals with compensated signs of alcoholic liver disease (GROUP-1) (with or without varices but no ascites), 30 individuals with decompensated alcoholic liver disease (GROUP-2) (ascites, jaundice, hepatic encephalopathy).

Control–apparentlyhealthyindividuals.

Inclusioncriteria: Patients with alcoholic liver disease diagnosedbyultrasoundorliverbiopsy.

Exclusioncriteria: Non-alcoholic liver disease patients, Hepatitis (virus), Liver autoimmune disorders, Wilson's disease and Alpha-1 antitrypsin deficiency are examples of genetic or metabolic liver illnesses, Patients with hepatocellular carcinoma, Inflammatory diseases such as pneumonia, ulcerative colitis, and Crohn's disease are examples.SAMPLECOLLECTION:5 mL of fasting venous blood sample was extracted from the patients' antecubital vein and collected in a simple vacutainer tube under aseptic conditions. Serum was separated by centrifugation at 3000 rpm for 15 minutes and kept at -200c for subsequent analysis.Kallistatin was tested using an enzyme linked immunosorbant assay (ELISA) kit from Bioassay Technology Laboratory, with a detection range of 0.5-200 ng/ml and a sensitivity of 0.22 ng/ml, according to the manufacturer's instructions. Das ELISA system (Reader A3 1851 & Washer 909) was used (Italy). The quantitative sandwich enzyme immunoassay method is used in the ELISA assay. Standards and samples (5,000-fold diluted serum) were incubated in a microtitrate coated with a polyclonal anti-human Kallistatin antibody in this experiment.Biotin-labeled antibody was added after incubation. The rest of the method (adding of streptavidin-HRP conjugate, substrate, and stop solution, etc.) followed the same pattern as the test described above. At a wavelength of 450nm, the absorbance of standards and samples was measured spectrophotometrically. The standard curve for standards was used to assess the concentration of Kallistatin. A dilution factor was applied to the findings (5,000).A microplate has been pre-coated with a monoclonal antibody specific for Kallistatin. The wells are pipetted with standards and samples, and any Kallistatin present is bound by the immobilised antibody.16After washing away any unattached compounds, the wells are filled with an enzyme-linked polyclonal antibody specific for Kallistatin. A substrate solution is added to the wells after a wash to eliminate any unbound antibody–enzyme reagent, and colour develops in proportion to the quantity of Kallistatin bound in the initial phase. The colour development is halted, and the colour intensity is assessed to determine the concentration of Kallistatin.The levels of total and direct bilirubin, AST, ALT, ALP, GGT, and albumin in the blood are measured using a typical automated analyzer (Beckman Coulter AU480). The Diazo Method of Pearlman and Lee was used to calculate total and direct bilirubin in the blood (an endpoint method). The Dynamic Extended Stability Modified IFCC Method was used to calculate serum alanine aminotransferase (SGPT) and serum aspartate aminotransferase (SGOT). The IFCC- Kinetic Method was used to calculate serum alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT). Bromocresol green, Endpoint assay was used to calculate serum albumin.

Statisticalanalysis: The statistical data was analysed using SPSS software. In patients with alcoholic liver disease and healthy controls, serum levels of total and direct bilirubin, AST, ALT, ALP, GGT, albumin, and Kallistatin are measured. The mean and standard deviation were computed. To compare the mean values, a students t test was used. The p value was computed. Significant is defined as a p value of less than 0.05. Highly significant is defined as a p value of less than 0.001.
The serum Kallistatin levels of compensated and decompensated alcoholic liver disease patients, as well as healthy controls, were compared using the ANOVA approach. Kallistatin was compared to ALP, AST, Albumin, GGT, Total, and Direct Bilirubin using the Pearson Spearman rank correlation method. The ROC (Receiver operating characteristic) Curve was used to determine the sensitivity of the biomarker Kallistatin in alcoholic liver disease.

RESULTS

S. Albumin,AST,ALT,ALP,GGT,Total bilirubin and Direct bilirubin, and Kallistatin serum levels in the alcoholic liver disease groups differed considerably from those in the control groups.

Table 1:Age Wise Distribution Of The Subjects

Age group	No.	Frequency
18-30 Yrs	28	31.1%
31- 50 Yrs	41	45.6%
51-70 Yrs	21	23.3%
Total	90	100.0%

There were 46 percent of subjects in the age category of 31-50 years, 31 percent in the age group of 18-30 years, and 23.3 percent in the age group of 51-70 years.

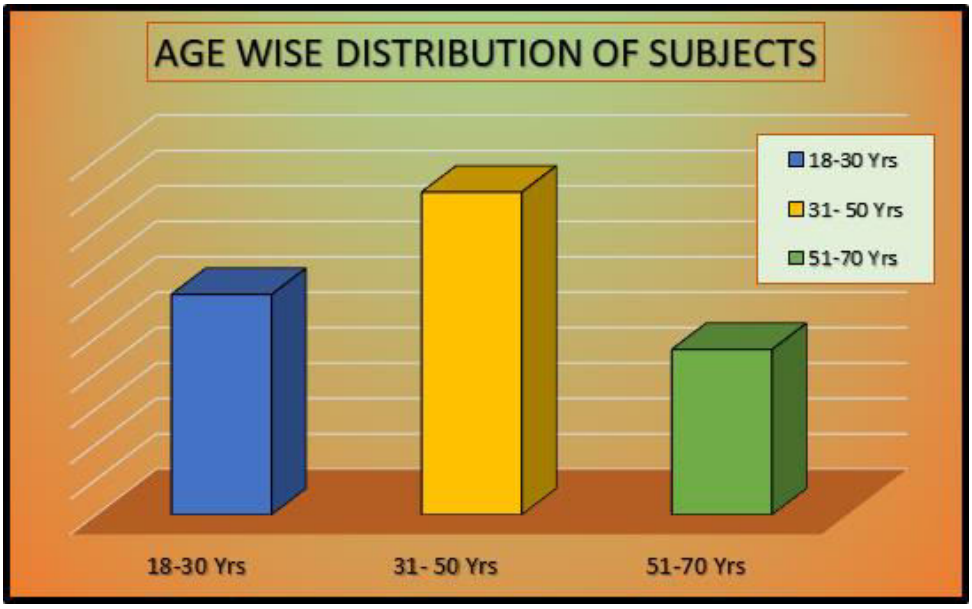


Figure 1: Age Wise Distribution Of The Subjects

Table 2: Mean Concentration of Kallistatin Between study groups

	Controls		Compensated liver disease		Decompensated liver disease	
	N=30		N=30		N=30	
Variable	Mean	SD	Mean	SD	Mean	SD
Kallistatin (µg/mL)	25.2	3.62	15.2	3.8	13	3.3

The mean Kallistatin value in compensated liver disease patients is 15.2 g/mL, 13.0 g/mL in decompensated liver disease cases, and 25.2 g/mL in controls.

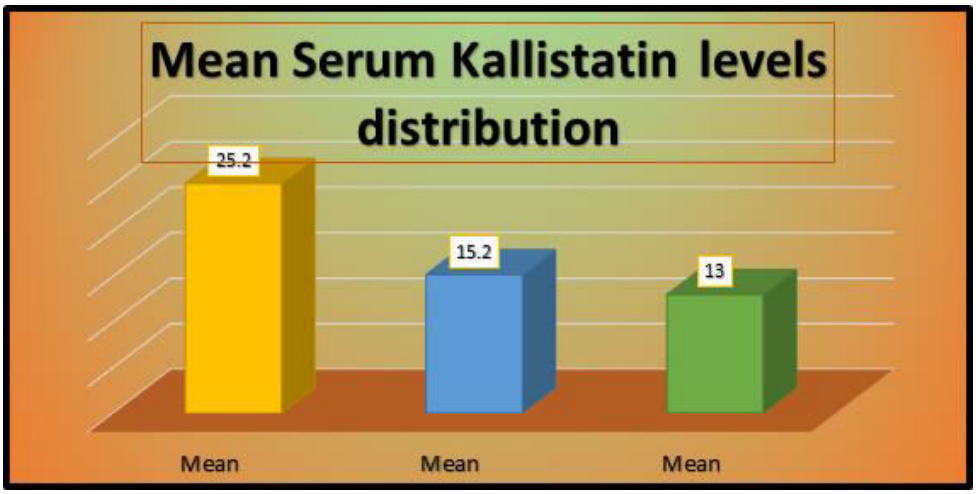


Figure 2: Mean concentration of Kalli statin Between study groups

Table 3: mean concentration of total and direct bilirubin and s. Albumin between study groups

	Compensated liver disease	Decompensated liver disease	Controls	P value
Total bilirubin(mg/dL)	1.96	5.26	0.92	0.0001
Direct bilirubin(mg/dL)	1.19	2.86	0.41	0.0001
Albumin(g/dL)	4.1	2.8	4.6	0.0001

Total and direct bilirubin, as well as S. Albumin, had mean values of 1.96, 1.19, and 4.1 in compensated liver disease patients, 5.26, 2.86, and 2.8 in decompensated liver disease cases, and 0.92,0.41 and 4.6 in controls, respectively.

Table 4: In the Study Groups, The Mean Concentration of Serum Hepatic Enzymes – SGOT, SGPT, AND ALP.

	Compensated	Decompensated	Controls	P value
SGOT(U/L)	93.2	102.2	24.1	0.058
SGPT(U/L)	43.2	45.2	26	0.18
GGT(U/L)	76.1	89.3	35.1	0.018

Serum hepatic enzymes SGOT, SGPT, and ALP have mean values of 93.2, 43.2, and 76.1 in compensated liver disease patients, 102.2, 45.2, and 89.3 in decompensated liver disease cases, and 24.1, 26 and 35.1 in controls, respectively.

Table 5: in the three study groups, the mean concentration of various analytes.

Parameter	Controls(n=30)	Compensated LD(n=30)	Decompensated LD(n=30)
Kallistatin (µg/mL)	25.2 +3.22	15.2 +3.00	13.0 +3.15
Total bilirubin(mg/dL)	0.92 + 0.88	1.96 +3.78	5.26+5.22
Direct bilirubin(mg/dL)	0.41 +0.56	1.19+2.27	2.86+3.52
SGOT(U/L)	22.8 +6.55	93.0 +224.06	102.3 +76.17
SGPT(U/L)	24.1 +4.65	39.2 +59.20	46.2 +42.51
ALP(U/L)	70.2 +14.88	86.3 +47.11	142.0 +18.2
GGT(U/L)	35.1+28.2	76.1+ 98.2	89.3 +68.22
Albumin(g/dL)	4.6 +0.39	4.1 +0.85	2.80 +0.46

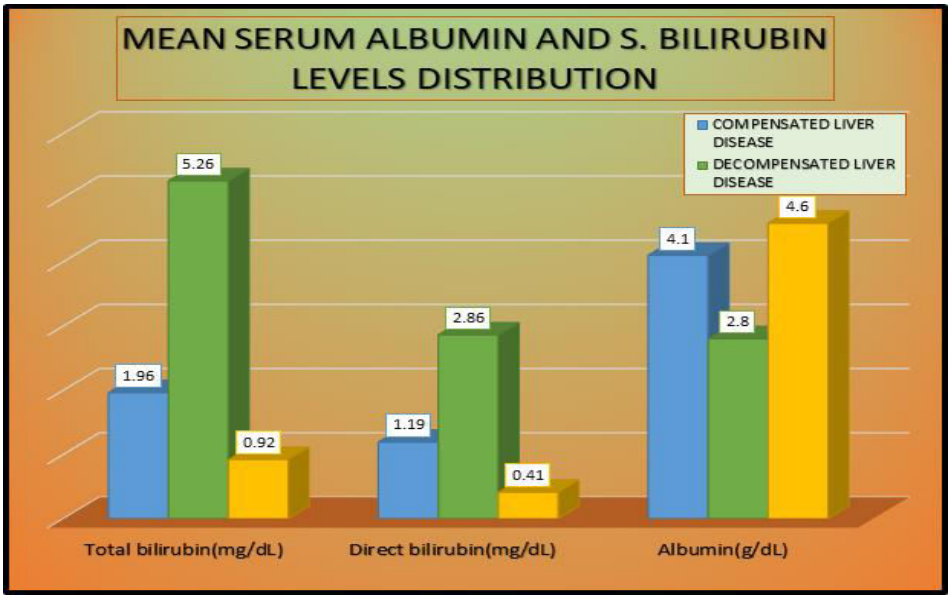


Figure 3: mean concentration of serum albumin and s. Bilirubin levels distribution

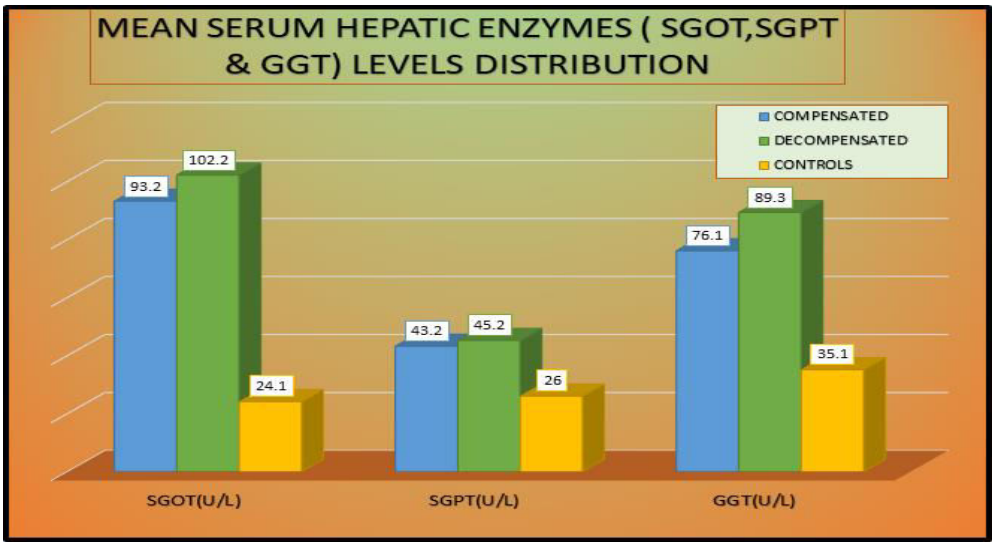


Figure 4: Distribution Of Mean Concentration Of Serum Hepatic Enzymes (SGOT,SGPT, & GGT).

Table 6: for various analytes in the three study groups, mean concentrations, SD, and SEM with p value.

Parameter	Controls(n=30)			Compensated LD(n=30)			Decompensated LD(n=30)			p Value
	Mean	SD	Std Error Of Mean	MEAN	SD	Std Error Of Mean	MEAN	SD	Std Error Of Mean	
Kallistatin (µg/mL)	25.2	3.22	0.59	15.2	3	0.58	13	3.15	0.62	0.0001
Total bilirubin(mg/dL)	0.92	0.88	0.18	1.96	3.78	0.77	5.26	5.22	0.88	0.0001
Direct bilirubin(mg/dL)	0.41	0.56	0.09	1.19	2.27	0.38	2.86	3.52	0.72	0.0001
SGOT(U/L)	22.8	6.55	1.2	93	224.06	41.2	102.3	76.17	14.2	0.0556
SGPT(U/L)	24.1	4.65	0.93	39.2	59.2	11.1	46.2	42.51	7.8	0.181
ALP(U/L)	70.2	14.88	1.3	86.3	47.11	8.9	142	18.2	11.2	0.015
GGT(U/L)	35.1	28.2	3.9	76.1	98.2	20.1	89.3	68.22	18.2	0.018
Albumin(g/dL)	4.6	0.39	0.06	4.1	0.85	0.2	2.8	0.46	0.2	0.0001

[Table 4] shows the (mean+ standard deviation) for all of the analytes studied in three groups: healthy controls, compensated liver disease patients, and decompensated liver disease patients. Data for total and direct bilirubin, SGOT, SGPT, GGT, ALP, albumin, and Kallistatin are displayed. After analysing all data, this study concludes that serum Kallistatin levels are significantly lower in compensated (14.50+3.0g/mL) and decompensated (12.34+3.15g/mL) liver disease compared to healthy controls (24.16+3.44g/mL).

Table 7: ANOVA was used to compare the results of several biochemical markers in three research groups.

Parameter	Controls(n=30)			CompensatedLD(n=30)			Decompensated LD(n=30)			p Value
	MEAN	SD	Std Error Of Mean	MEAN	SD	Std Error Of Mean	MEAN	SD	Std Error Of Mean	
Kallistatin (µg/mL)	25.2	3.22	0.59	15.2	3	0.58	13	3.15	0.62	0.001 **
Total bilirubin(mg/dL)	0.92	0.88	0.18	1.96	3.78	0.77	5.26	5.22	0.88	0.0001 ***
Direct bilirubin(mg/dL)	0.41		0.09	1.19	2.27	0.38	2.86	3.52	0.72	0.0001-***
SGOT(U/L)	22.8	6.55	1.2	93	224.06	41.2	102.3	76.17	14.2	0.0556-NS
SGPT(U/L)	24.1	4.65	0.93	39.2	59.2	11.1	46.2	42.51	7.8	0.181 - NS
ALP(U/L)	70.2	14.88	1.3	86.3	47.11	8.9	142	18.2	11.2	0.015
GGT(U/L)	35.1	28.2	3.9	76.1	98.2	20.1	89.3	68.22	18.2	0.018 - *
Albumin(g/dL)	4.6	0.39	0.06	4.1	0.85	0.2	2.8	0.46	0.2	0.0001-***

**HIGHLYSIGNIFICANTAT1%LEVEL.
*NOT SIGNIFICANTS-SIGNIFICANT
HS-HIGHLYSIGNIFICANTNS-NOTSIGNIFICANT

[TABLE 5] compares the values of biochemical parameters in three research groups using ANOVA. Kallistatin, total and direct bilirubin, albumin, and GGT all had statistically significant p values. For SGOT and SGPT, p values are shown to be insignificant. When compared to controls (25.2 +3.22 µg/mL), there is a substantial drop in mean serum Kallistatin levels in the compensated group of 15.2 +3.0g/mL and the decompensated group of 13.0 +3.15 µg/mL, with a very significant p value of 0.001.

Table 8: Various Parameters of Alcoholic Liver Disease And Serum Kallistatin Levels Pearson Correlation (R) And Spearman Rank Correlation (p).

Kallistatin (µg/mL)	**Total bilirubin(mg/dL)**	**Direct bilirubin(mg/dL)**	**SGOT(U/L)**	**SGPT(U/L)**	**ALP(U/L)**	**GGT(U/L)**	**Albumin(g/dL)**
Pearson correlation							
r value	-0.31	-0.22	-0.27	-0.22	-0.48	-0.33	0.62
p value	0.0001 ***	0.0001 ***	0.0556-NS	0.181 -NS	0.015	0.018 -*	0.0001- ***
Spearman rank correlation							
r value	-0.62	-0.48	-0.7	-0.5	-0.52	-0.59	0.62
p value	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**

The Pearson and Spearman correlation between blood Kallistatin levels and other liver function tests is shown in [Table 6]. Serum Kallistatin levels and serum albumin levels were shown to have a strong positive connectionin patients with alcoholic liver disease. Total bilirubin, direct bilirubin, AST, ALT, ALP, and GGT all have a strong negative relationship with serum Kallistatin levels.

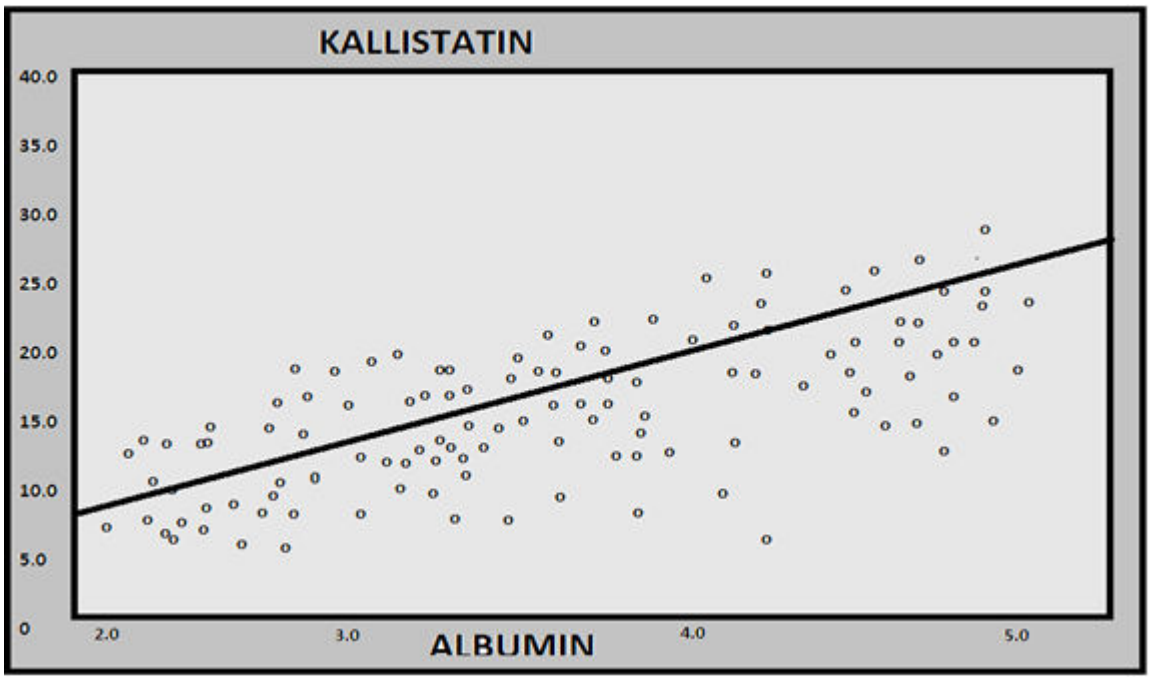


Figure 6: albumin levels and serum kallistatin levels in patients with alcoholic liver disease.

With a r value of 0.56 and a p value of 0.001, [Figure 6] reveals a strong positive connection between serum Kallistatin levels (highly significant).

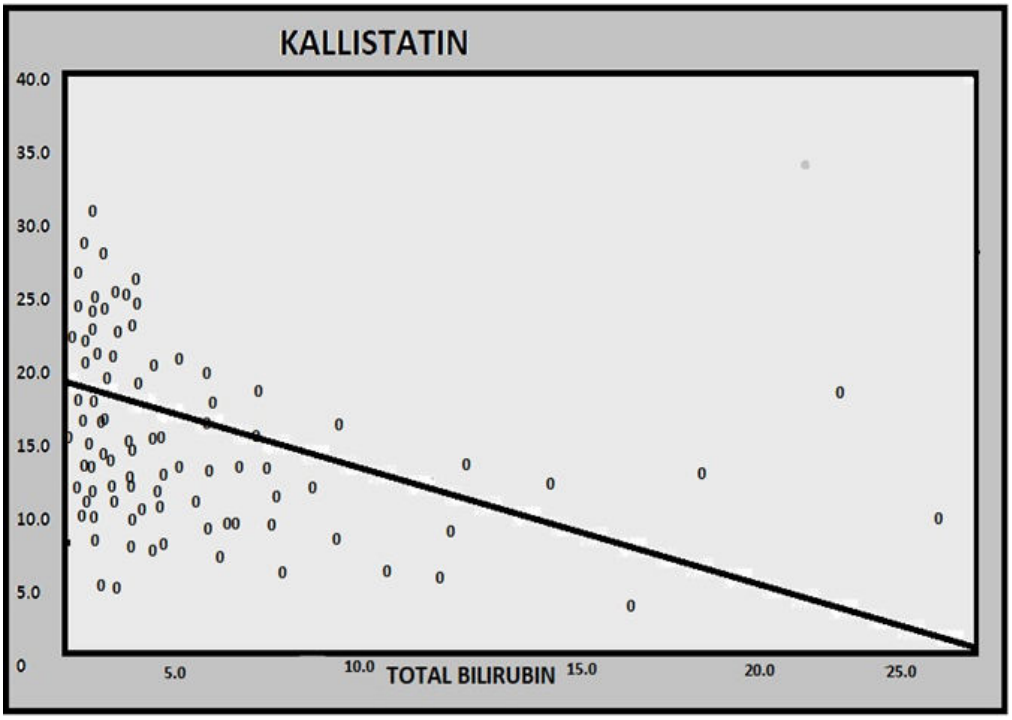


Figure 7: Correlation of Serum Kallistatin And Total Bilirubin In Alcoholic Liver Disease Patients.

With a r value of 0.28 and a p0.001 value, [Figure 7] reveals a negative connection between serum Kallistatin levels and total bilirubin levels (highly significant).
In patients with alcoholic liver disease, there is a correlation between serum kallistatin levels and direct bilirubin levels [Figure 8].
With a r value of 0.29 and a p value of 0.001, [Figure 9] reveals a negative association between serum Kallistatin levels and direct billirubin levels (highly significant).

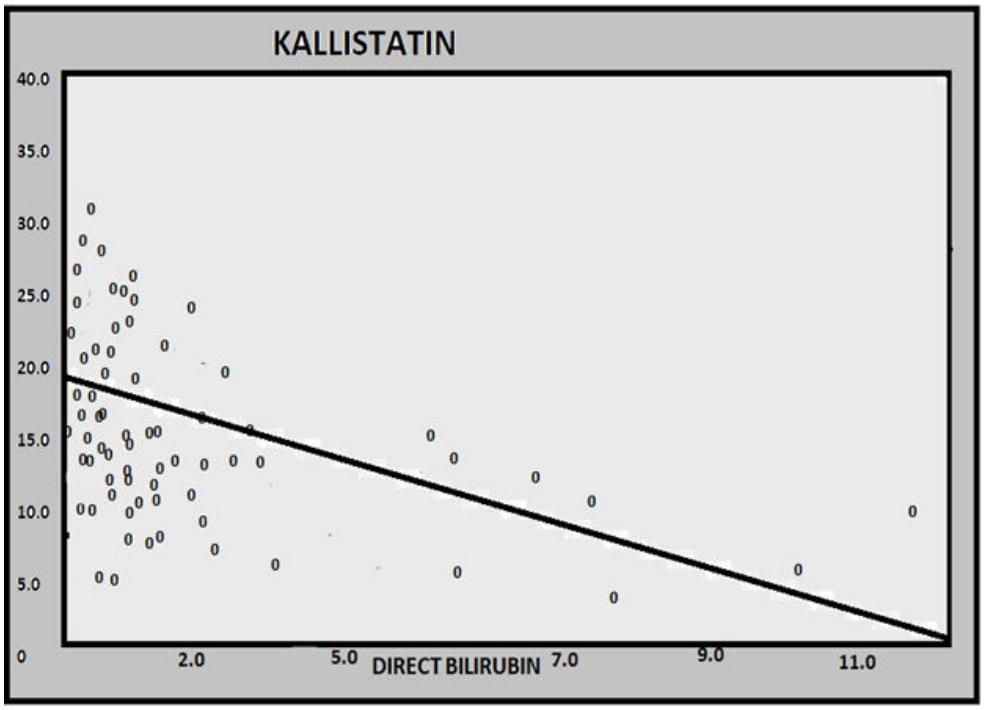


Figure 10: relationship between serum kallistatin levels and alkaline phosphatase levels in alcoholic liver disease patients.

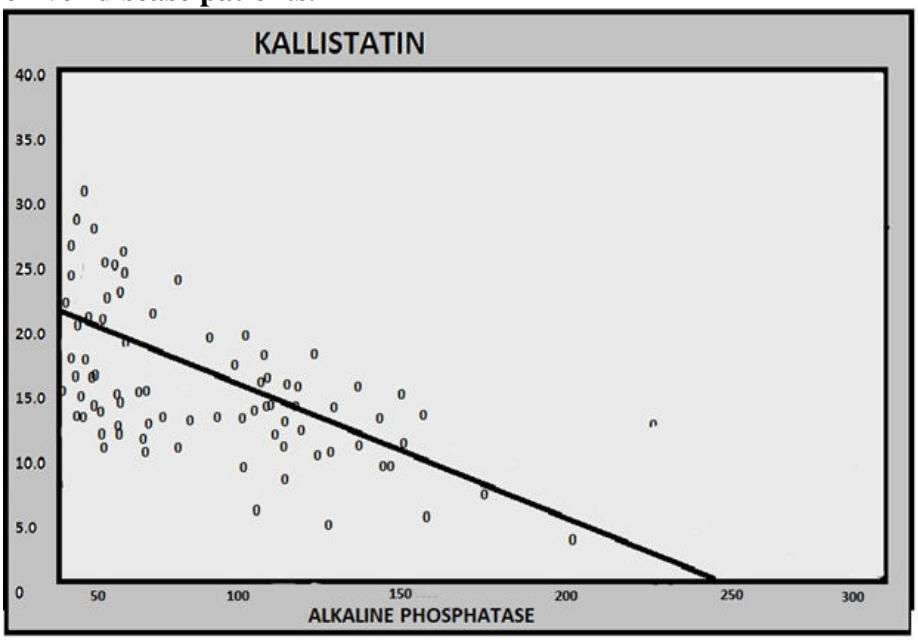
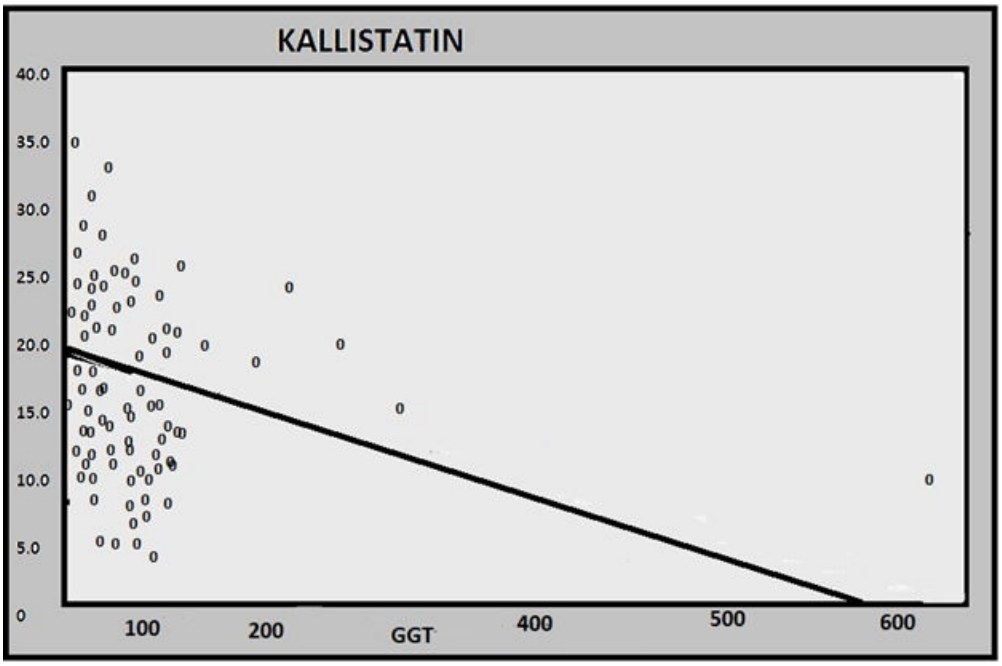


Figure 11: Correlation between serum kallistatin Levels and GGT Levelsinpatientswithalcoholicliverdisease

With a r value of 0.49 and a p value of 0.001, Figure 9 reveals a negative connection between serum Kallistatin levels and alkaline phosphatase levels (highly significant).



With a r value of - 0.27 and a p value of 0.003, Figure 10 reveals a negative association between serum Kallistatin levels and GGT levels (significant).

Table 9: BASEDONROC

Variable	
Area Under Curve(AUC)	0.922
Optimal cut of value	20.22µg/mL
Sensitivity	93.65 %
Specificity	96.30 %
Positive Predictive value	98.33 %
Negative Predictive value	86.67 %

Receiver Operating Characteristic curve: The x-axis represents specificity, while the y-axis represents sensitivity. The area under the curve was 0.922, with a cut-off value of 20.22g/mL, demonstrating that Kallistatin can be a useful non-invasive biomarker for identifying alcoholic liver disease. The sensitivity, specificity, positive and negative predictive values are 93.65%, 96.30%, 98.33%, and 86.67%, respectively.

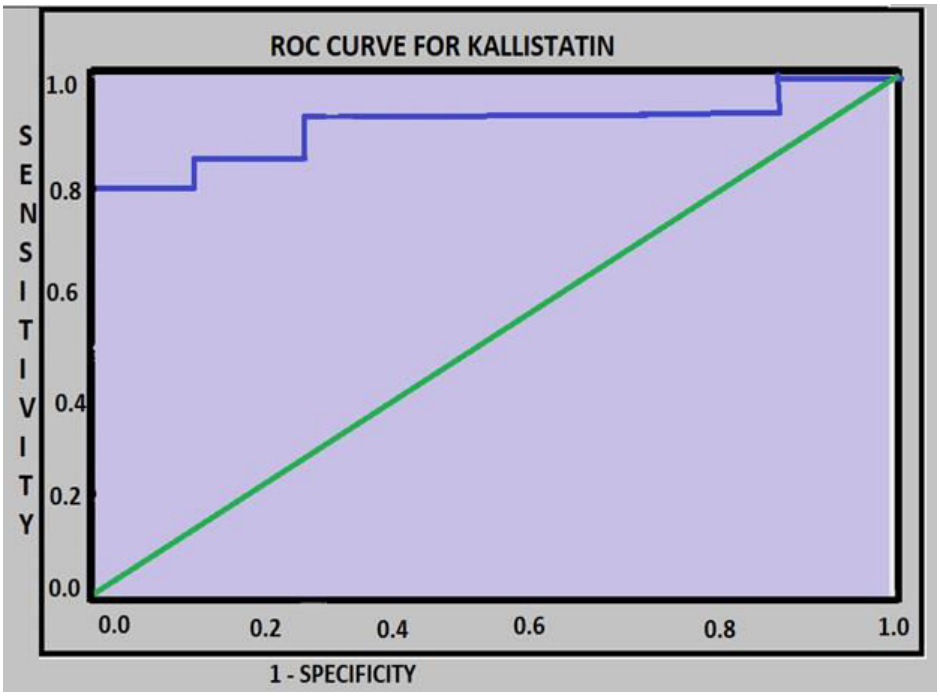


Figure 12: Receiver Operating Characteristic curve proves that Kallistatin can be a good non-invasive biomarker for diagnosing alcoholic liver disease.

DISCUSSION

Alcoholism is a widespread societal stigma in which moderate alcohol drinking appears to be normal. Organ harm begins when drinking becomes a regular and frequent habit. Alcoholism frequently affects the liver. Because the liver has a considerable reserve capacity, the patient seems healthy at first, but as the liver injury advances, its functions are clearly affected, as seen by a drop in albumin production.

In a review research, ultrasound-based categorization of liver damage was shown to be more accurate than liver biopsy. They found that 81.48 percent of compensated liver disease and 91.7 percent of decompensated liver disease could be detected. Nearly half of all individuals with liver disease are diagnosed in the compensated stage . With corrected characteristics, they can survive for ten to twelve years. Patients with compensated characteristics may develop esophageal varices, but mortality is decreased to one year once de-compensated signs, such as portal hypertension, ascites, and encephalopathy, are present.

Hepatocellular malignancy is thought to be preceded by decompensated liver disease. The liver is responsible for a wide range of functions. A sequence of diseases in other organ systems causes functional impairment. A single test will not be able to detect liver damage or track the evolution of liver disease. Minor liver damage cannot be identified early on because compensatory mechanisms make the liver functions seem normal.

Some tests may indicate liver damage, but they are ineffective in determining illness prognosis and vice versa.

Cirrhosis of the liver is the most common long-term outcome of all chronic liver disorders. In individuals with cirrhosis associated with chronic hepatitis B or C infection, the yearly incidence rates of liver failure (decompensation), HCC, and mortality are around 4%, 3%, and 3%, respectively.

In this work, kallistatin, a non-invasive biomarker, is used to demonstrate its diagnostic sensitivity in detecting liver dysfunction at an early stage and to track development as it progresses to a susceptible stage that causes irreparable liver damage. Using Kallistatin levels to track the evolution of liver damage caused by alcohol, alcoholic liver disease has been divided into two categories: compensated liver disease and de-compensated liver disease.

This study contains a control group of 30 people and a study group of 60 people, all of whom are in the same age range. Because females were not exposed to alcoholic history or presented with any evident signs in ultrasonography abdomen, indicative of alcoholic liver disease, both the control and research groups were made up entirely of males. Indian girls have a tradition of not disclosing their drinking past in social situations. This could not be deemed a research constraint because other investigations from various countries confirmed the findings that Kallistatin levels did not differ significantly between gender and age categories.

Depending on the technique used, the values of all the parameters in this study were determined to be within the reference range stated in various reviews or the kit insert values for reference range. In this study, meanserumKallistatin levelswere(25.22 ±3.62µg/mL),(15.2 ±3.8 µg/mL)and(13±3.3µg/mL) in Control group,compensatedgroup and decompensatedgroup respectively , which is closer to the Chao J et al study's reference range of 22.1+3.5 µg/mL . When alcoholic liver disease progresses to the decompensated stage, numerous analytes are impacted, including total and direct bilirubin, albumin, SGOT, SGPT, ALP, and GGT. The substrates and enzymes are tested using kit procedures. These methods are based on IFCC processes that have been modified, as well as enzymatic and kinetic approaches.

Alcoholicliverdiseasepatientsshowmarkedly elevatedlevelsofconjugatedbilirubinlevel in blood. In thisstudy, [Figure 2] showed a markedlyelevatedlevelsoftotalanddirectbilirubinindecompensatedliverdiseasethancompensate dliverdiseaseSynthesisofalbumindecreases in cases of alcoholic liver disease oftenassociatedwithmalnutrition.

Although Kallistatin is found in many human organs, several studies have demonstrated that the liver is the primary location of Kallistatin production and release, explaining the much lower levels of serum Kallistatin found in individuals with hepatic cirrhosis.

Kallistatin is a negative acutephase protein, the concentration of which decreases during inflammatory processes (e.g. significantly reduced kallistatin levels were seen in the plasma of a patient with sepsis). In cases of cirrhosis-associated liver damage, its serumlevels decrease. Reduced production of kallistatin results from liver damage, especially impaired synthetic function,and correlates with the stage of liver cirrhosis.

When cirrhotic patients, particularly those who were decompensated, were compared to normal controls and HCC patients, Kallistatin serum levels were considerably lower. These findings were in line with those of Cheng et al., who found that serum Kallistatin levels in

patients with hepatic cirrhosis were considerably lower than those in healthy controls, indicating a strong link between serum Kallistatin levels and the severity of hepatic illness. They came to the conclusion that serum Kallistatin levels might be used as a biomarker for detecting hepatic cirrhosis and gradual loss of liver function. A significant decrease in kallistatin levels (7.272.5 mg/mL, Po0.001) was also found in plasma samples from nine patients with liver disease in a study by Chao et al.[11]

Cheng et al.[17] discovered that an optimal cut-off value for LC diagnosis was 21.64 µg/mL of Kallistatin, with 87.1 percent sensitivity, 74.2 percent specificity, 91 percent positive predictive value, and 62.3 percent negative predictive value, respectively, but with insignificant values in predicting decompansation and hepatocellular carcinoma. On the other hand, we discovered that a Kallistatin serum level of 20.22g/mL, demonstrating that Kallistatin can be a useful non-invasive biomarker for identifying alcoholic liver disease. The sensitivity, specificity, positive and negative predictive values are 93.65%, 96.30%, 98.33%, and 86.67%, respectively.

These differences may be related to our patients' inclusion criteria, as we could choose relatively homogenous chronic liver disease patients diagnosed as chronic hepatitis C infected patients and subdivided into compensated, decompensated, and hepatocellular carcinoma groups, whereas Cheng et al.[17] chose compensated, decompensated, and hepatocellular carcinoma groups with different etiologies (viral hepatitis C and B, alcoholic hepatitis and uncertain etiology).

Hepatic cells contain a large quantity of SGOT and SGPT. This enzyme seeps out of cells and into circulation whenever the liver is damaged by alcohol or pollutants. They lack sensitivity, while being strong indicators of liver injury. As shown in earlier research, there is no statistically significant difference in the mean values of SGOT and SGPT in patients and controls, as shown in [Table 4]. As shown in table 4 (SGOT level of 102.3 U/L and ALT level of 46.2 U/L in decompensated LD patients), aspartate transaminases levels are much higher than alanine transaminases. This is owing to the fact that AST is distributed more widely throughout our body's tissues than ALT. Although serum alkaline phosphatase is derived from the outer cell membrane of the liver, it performs fundamentally distinct functions. ALP activity is helpful in determining the cause and amount of liver injury.

Table 4 shows that serum alkaline phosphatase levels were higher in compensated alcoholic liver disease (86.3 + 47.11 U/L) and decompensated alcoholic liver disease (142 +18.2 U/L) than healthy controls (70.2 +14.88 U/L) in this research. The enzyme gamma glutamyl transferase is found in the biliary canaliculi. Longer periods of alcohol use produce liver injury and an increase in GGT levels. GGT levels do not rise in the early stages of alcoholic liver disease or in patients returning for follow-up following therapy, according to a research by Krastev et al 18. In Table 7, the comparison of GGT levels between control, compensated, and decompensated liver disease reveals statistical significance with a p value of 0.05, as in the research by S.Orlowskin et al.[19]

This rise might be attributable to microsomal induction and hepatic cell damage. Markers like AST and ALT have low sensitivity, specificity, or both, according to the literature. Some markers, such as GGT, are not very specific. The goal of this study is to assess the function of Kallistatin as a non-invasive biomarker in the diagnosis of alcoholic liver disease, as well as its use in detecting disease severity and comparing serum Kallistatin levels in seemingly healthy people. Pearson and Spearman rank correlations [Table 7] were used to examine the positive and negative association between serum Kallistatin levels and the other variables in the research. Figure 6 shows that serum Kallistatin levels were positively connected with serum albumin (r=0.56 and p value 0.001), but negatively correlated with GGT levels (r=-0.27 and p value0.03). In addition, serum Kallistatin is inversely linked with ALP in figure 9, with a r=0.49 and a p value of 0.001. [Figures 7 and 8] indicate negative relationships

between serum Kallistatin and total bilirubin (r=-0.28 and p value 0.001) and direct bilirubin (r=-0.29 and p value 0.001).

The serum Kallistatin assay had a highly significant p value of 0.001 in the ANOVA research in [Table 7]. According to the findings, albumin, like serum Kallistatin levels, is a highly deciding variable with a very significant p value of 0.001. [Table 9] shows that Kallistatin can be an useful non-invasive biomarker for identifying alcoholic liver disease, with a sensitivity of 93.65% and specificity of 96.30 percent. The positive predictive value is 98.33%, while the negative predictive value is 86.67 percent. The best cut-off value is 20.22µg/mL, and the area under the curve is 0.922. Simultaneous assessment of most of the analytes in the liver panel is one of the study's main pillars.Serum albumin levels assess synthetic function, serum total and direct bilirubin levels assess excretory function, and AST,ALT,ALP, and GGT levels assess enzyme profile. This study looks at the association between serum Kallistatin levels and other biochemical markers.

CONCLUSION

Sixty cases of alcoholic liver disease, as determined by ultrasonography abdomen and clinical history, were included in this investigation. Thirty age and gender matched controls with normal ultrasonography abdomen and no history of alcohol consumption were chosen. In sixty patients and thirty controls, several parameters such as total and direct bilirubin, albumin, SGOT, SGPT, ALP, and GGT were tested. SerumKallistatin was discovered to be a useful indicator for detecting early liver damage caused by alcohol use in this investigation. As liver damage progresses, serum Kallistatin levels fall. As a result, alcoholic liver disease-related mortality rises.

The levels of serum kallistatin were favourably connected with albumin levels and negatively correlated with total and direct bilirubin, ALP, and GGT. These findings suggest that serum Kallistatin levels may be an important and protective factor in the prevention of alcoholic liver damage. This research points to a potential for therapeutic intervention to begin sooner based on blood Kallistatin levels. In individuals with alcoholic liver disease, serum Kallistatin levels can be estimated as part of standard examinations for liver function testing.

Limitations of the Study:

A small sample size obtained from a single medical institution may deprive us of the exact significant values that would be more visible if collected from a variety of hospitals. The study's small subgroups make it difficult to establish a link between specific co-morbidities and alcoholic liver disease. A follow-up research may have confirmed our findings and demonstrated the benefits of Kallistatin as a novel non-invasive biomarker for detecting liver damage and its treatment benefits.

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