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Evaluation of Serum Kallistatin Levels in Cases of Alcoholic Liver Disease

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ABSTRACT: INTRODUCTION: Kallistatin, a human serine proteinase inhibitor is synthesised by liver**.** The presentstudyevaluated the role of Kallistatin as a non-invasive marker in the diagnosis of alcoholic liver disease (ALD), its usefulness in correlation with disease severity and to compare serum Kallistatin levels in ALD patients with healthy controls.**MATERIALS AND METHODS:** Sixty Alcoholic liver disease patients (divided into two groups based on compensated and decompensated features) and thirty healthy controls were included in this study.5 mL of fasting venous blood was collected and serum separated to measure Total and direct bilirubin, AST, ALT, ALP, GGT, albumin using conventional automated analyzerand Kallistatin levels by Enzyme Linked Immunosorbent Assay.The results were statistically analysed using t- test, Pearson Spearman rank correlation, Anova and Receiver operating characteristic (ROC) Curve (SPSS version 16.0 software)**RESULTS:**Mean value of kallistatin for control is 24.16µg/mL and case is13. 42µg/mL.Statistically significant p value was obtained (p value< 0.001). kallistatin levels decreases as the liver damage increases.Serum levels of AST, ALT, ALP, GGT wereraised andalbumin decreased in ALD patients and was statistically significant. The study shows a significant positive correlation ($p<0.05$) between Serum kallistatin and albumin levels, and significant negative correlation ($p<0.05$)between Serum kallistatin and GGT levels.ANOVA revealedstatistically significant p value <0.001 and ROC with best cut off value 0.922 for kallistatin. **CONCLUSION:** The present study proves Kallistatin as a reliable diagnostic non-invasive biomarker in the early diagnosisof ALD patients.

KEY WORDS: SerumKallistatin, Alcoholic Liver disease, Biomarker, Alcoholism.

I. INTRODUCTION:

Alcoholism is one of the most common causes of liver disease all over the world. Liver injury begins with simple steatosis due to alcoholism, followed by alcoholic hepatitis, fibrosis of liver, ending eventually in cirrhosis [1, 2]. Risk factors for liver damage are chronic alcohol intake, obesity, genetic factors and viral hepatitis[3]. Liver injury occurs based on the quantity, duration of alcohol intake and drinking pattern [4]

Alcoholism is a major socioeconomic problem often diagnosed by reporting of the patient himself, but a physician should consider this with high suspicion. Mostly, alcoholics mimic healthy persons, when they reach the physician. At a later phase when the person started with complaints due to alcohol or other reasons, organ damage would have occurred due to alcoholism[5]. Excessive intake of alcohol accounts for 15-20% of visits of patients in primary health care centres and 20-30% of hospitalised patients[6].

Alcoholics are safer to be identified at an early stage so that the deleterious effects of alcohol can be prevented earlier and therapeutic intervention along with prognosis will also be better in alcoholic liver disease[7,8]. A study showed 14% rise in cirrhosis patients for each 1000 mL alcohol intake irrespective of the beverage consumed[9]. WHO showed an estimate of morbidity and mortality as a consequence of alcoholic liver disease being higher in developed countries (9.2% of DALY) than developing countries[10,11]. Sikkim shows a highest average of 51% and after alcohol withdrawal, the relapse on an average is 35%[12].

Alcoholic liver disease can be characterized byAlcoholic fatty liver, Alcoholichepatitis and Cirrhosis.[17,18]. Pathological features begins with simple steatosis[19]. After chronic alcohol intake, there will be a collection of fatty acids in hepatic cells leading to triglyceride

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accumulation triggering fatty liver[20]. Alcoholic liver disease presents features similar to viral hepatitis and hepatoma[3]. Alcoholism with hepatitis B and C worsens morbidity leading to chronic viral hepatitis, cirrhosis and death due to hepatocellular malignancy[17,18,19]. In alcoholic liver disease, liver damage shows hepatocellular necrosis and fibrinogenesis in stellate cells due to oxidative stress[16]. Liver damage occurs due to cellular immune response against hepatocytes affected by viral infection[21]. Malnutrition and depression causes high mortality in patients with alcoholic liver disease[22].

However, if problems due to alcohol intake are diagnosed earlier, a physician can easily prevent further progression to alcoholic liver disease. Recently, Biomarkers play an important role in detecting alcohol induced liver disease at an early stage, motivating patient for abstinence from alcohol and monitor progression while patient is on treatment[23].

Kallistatin is a kallikrein binding protein present in humans encoded by serpinA4 gene[24]. It is a medium sized protein belonging to serpin family. The word SERPIN indicates Serine Protease inhibitor but it is a misnomer. All proteins of serpin family are not serine protease inhibitors and vice versa[24]. It is a negative acute phase reactant[25]. Kallistatin combines with tissue kallikrein to form a covalent bond complex and decreases during sepsis[24]. Kallistatin is a unique serine proteinase inhibitor which opposes inflammatory action.Liver synthesize and secretes kallistatin[25].kallistatin levels decreases as the liver damage increases inalcoholics, indicating the progression of cirrhosis. Therefore, decreased kallistatinlevels is considered as an alcoholic liver disease risk factor.

Kallistatin is a plasma protein which binds with tissue kallikrein and decreases in its levels as liver damage progresses due to alcoholism[26]Kallistatin plays an important role against apoptosis[27]. It has anti angiogenic, antitumor and anti-oxidant properties[28,29]. Therefore this study was undertaken to evaluate the role of kallistatin as a non-invasive marker in diagnosing alcoholic liver disease and assessing the severity in liver disease among patients admitted in Rajiv Gandhi Government General Hospital (RGGGH), Chennai.

AIM: To evaluate the role of kallistatin as a non-invasive marker in the diagnosis of alcoholic liver disease.

OBJECTIVE:

1)To assess the disease severity with serum kallistatin levels.
2)To compare serum kallistatin levels in alcoholic liver disease patients with apparently healthy individuals.

To fulfil the aim of the study, serum kallistatin levels are assessed in control groups and by dividing the cases into two groups viz., compensated or asymptomatic group and decompensated or progressive group. The other parameters assessed are AST, ALT, ALP, GGT, Serum total and direct bilirubin and albumin.

II. MATERIALS AND METHODS:

This study was conducted during the period of July 2016-December2016 as a case control study in the Institute of Biochemistry &Department of MedicalGastroenterology, Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai – 3

STUDY POPULATION

The study was conducted after getting ethical committee approval. The study is comprised of a total number of 90 human subjects. Controls were recruited from outpatient department during their visit for non-hepatic causes. 30 apparently healthy individuals, with history of total abstinence from alcohol and normal ultrasound abdomen were selected. 60 cases were selected from gastroenterology department, Rajiv Gandhi Government General Hospital (RGGGH), Chennai. Consent was taken from all the subjects as well as the controls. **CASES**- Patients with alcoholic liver disease. Patients were grouped into two – based on compensated and decompensated features.
GROUP-1: 30 alcoholic liver disease patients with compensated features (with or without varices but no ascites)
GROUP-2: 30 alcoholic liver disease patients with decompensated features (ascites, jaundice, hepatic encephalopathy)

- **CONTROL** –apparently healthy individuals

INCLUSION CRITERIA:

- Patients with alcoholic liver disease diagnosed by ultrasound or liver biopsy.

EXCLUSION CRITERIA:

- Patients with non- alcoholic liver disease.
- Viral hepatitis.
- Autoimmune liver diseases.
- Genetic or Metabolic liver diseases like Wilson's disease, Alpha-1 antitrypsin deficiency.

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- Hepatocellular carcinoma patients.
- Inflammatory conditions like pneumonia, ulcerative colitis and Crohn's disease.

SAMPLE COLLECTION:

5 mL of venous blood in fasting was drawn from antecubital vein of patients and collected in a plain vacutainer tube under aseptic precautions and serum separated by centrifugationat 3000rpmfor 15 minutes and stored at -20^{0}c for further analysis.

Quantitative measurement of human serum kallistatinlevels are done by Enzyme Linked Immunosorbent Assay (Bioassay Technology Laboratory, Shangai, China.), a Sandwich Immunoassay Technology using antibodies. Serum total and direct bilirubin, AST, ALT, ALP, GGTand albuminare estimated using conventional automated (Beckman coulter AU480) analyzer.Serum Total and Direct Bilirubin was estimated by Diazo Method of Pearlman and Lee (an endpoint method). Serum alanine aminotransferase (SGPT) and serum aspartate aminotransferase (SGOT) was estimated by Dynamic Extended Stability Modified IFCC Method. serum alkaline phosphatase (ALP)and gamma glutamyl transferase (GGT)was estimated by IFCC- Kinetic Method. Serum albumin was estimated by Bromocresol green, Endpoint assay.

III. RESULTS ANDSTATISTICAL ANALYSIS:

The serum levels of parameters including Albumin,AST,ALT,ALP,GGT,Total and Direct bilirubin and kallistatin in the alcoholic liver disease groups were significantly different from those of control groups

STATISTICAL ANALYSIS

SPSS (Statistical Package for Social Science) version 16.0 software was used for analysing statistical data. Serum levels of Total andDirect bilirubin, AST, ALT, ALP, GGT, albumin and kallistatin are done in patients with alcoholic liver disease and healthy controls. Mean and Standard deviation are calculated. Students' "t" test was done to compare the mean values. p value was calculated. A p value of less than 0.05 is considered to be significant. A p value of less than 0.01 is considered to be highly significant. ANOVA was done to compare serum kallistatin levels between compensated and decompensated alcoholic liver disease cases and healthy controls. A p value <0.05 is considered to be significant.Pearson Spearman rank correlation of kallistatin with other variables like ALP, AST, Albumin, GGT, Total and Direct bilirubin was done. Receiver operating characteristic (ROC) Curve was done to assess sensitivity and specificity of kallistatin. Area undercurve (AUC) is useful for finding out the expected cases of alcoholic liver disease. Best cut off values are obtained for serum kallistatin level.

TABLE 1
MEAN CONCENTRATION OF KALLISTATIN BETWEEN STUDY GROUPS

Variable	Control			Compensated			Decompensated			p-value
	N=30			N=30			N=30			
	Mean	Sd	SEM	Mean	Sd	SEM	Mean	Sd	SEM	
Kallistatin(µg/mL)	24.16	3.44	0.63	14.50	3.00	0.55	12.34	3.15	0.58	<0.001

The mean kallistatin value of compensated LD cases is14.50, decompensated LD cases is 12.34 and that of controls is 24.16 and the p value is <0.001 which is highly significant.

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FIGURE 1

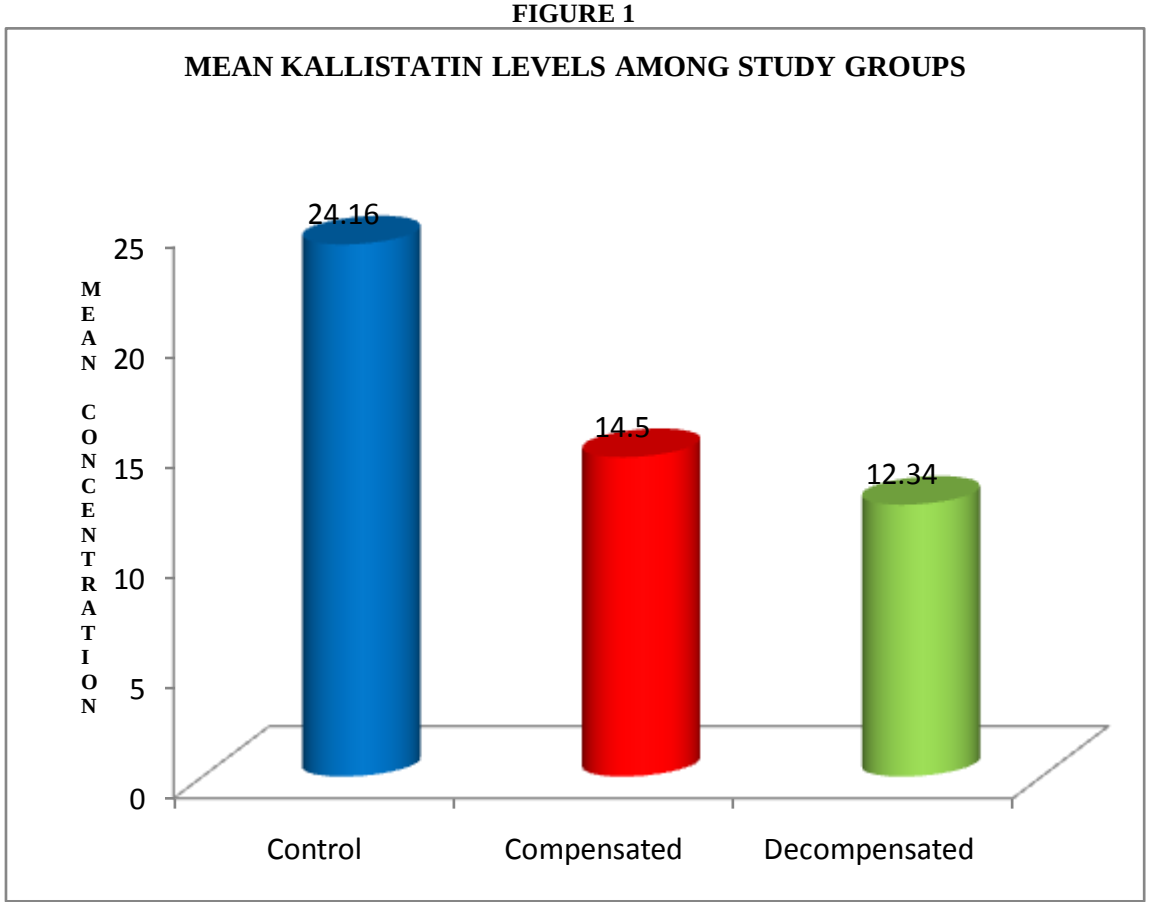

TABLE 2
MEAN CONCENTRATION OF TOTAL AND DIRECT BILIRUBIN BETWEEN STUDY GROUPS

	COMPENSATED	DECOMPENSATED	CONTROLS	p VALUE
Total bilirubin(mg/dL)	1.96	5.26	0.92	0.000
Direct bilirubin(mg/dL)	1.19	2.86	0.41	0.001

The mean value of total and direct bilirubin in compensated LD cases is 1.96 and 1.19, decompensated LD cases is 5.26 and 2.86 and that of controls is 0.92 and 0.41 respectively. p value is <0.001 is highly significant.

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FIGURE 2

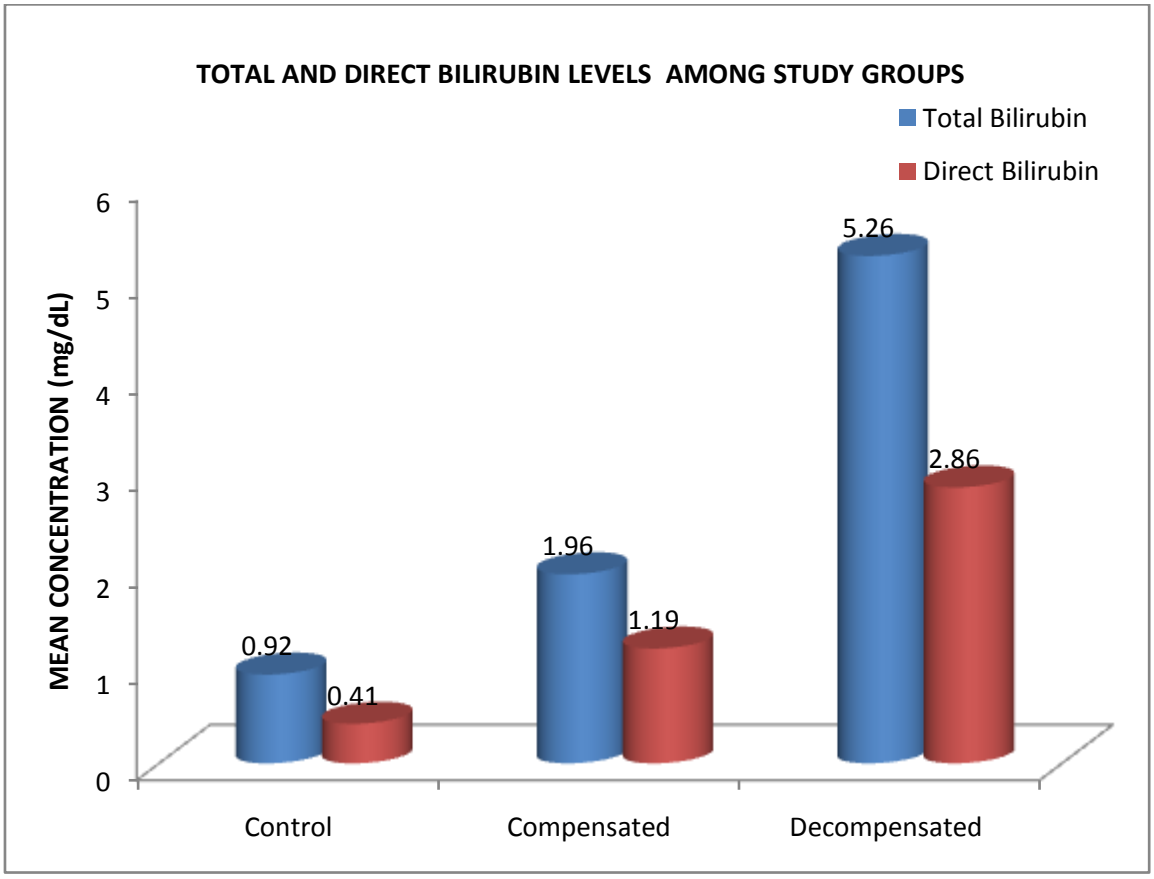

TABLE3

MEAN CONCENTRATION OF ALBUMIN BETWEEN STUDY GROUPS

	COMPENSATED	DECOMPENSATED	CONTROL	P VALUE
Albumin(g/dL)	3.85	2.7	4.28	0.000

The mean value of albumin in compensated LD cases is 3.85, decompensated LD cases is 2.7and that of controls is4.28. p value is <0.001 is highly significant

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Figure 3

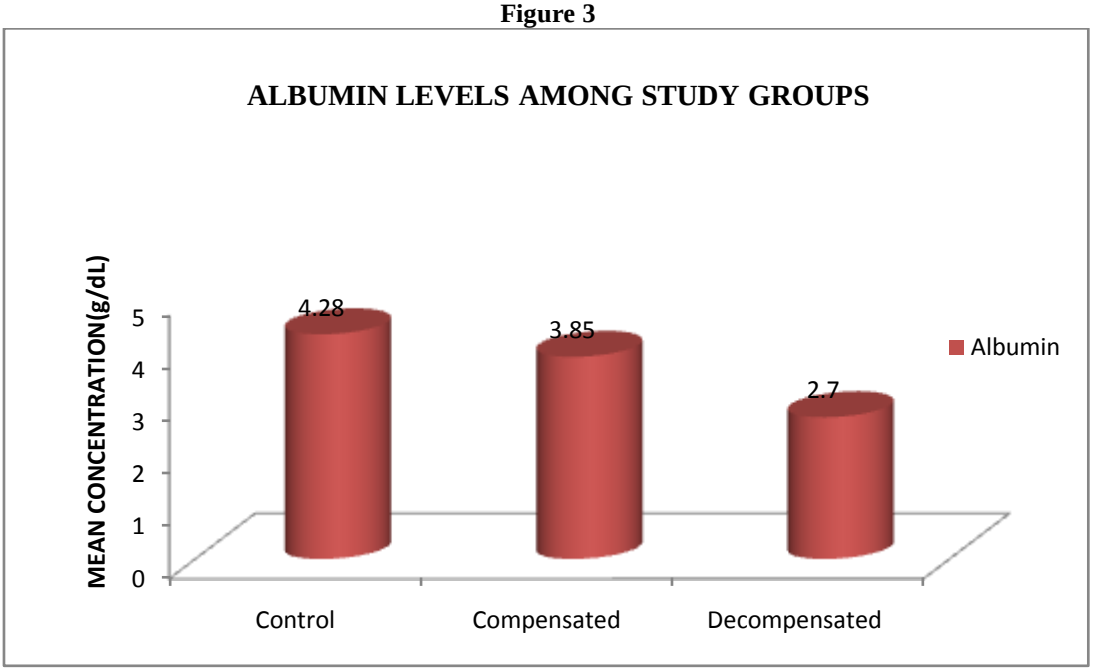

TABLE 4
MEAN CONCENTRATIONS OF SGOT AND SGPT BETWEEN STUDY GROUPS

	COMPENSATED	DECOMPENSATED	CONTROLS	p VALUE
SGOT(U/L)	91.73	100.87	23.2	0.06
SGPT(U/L)	41.47	44.6	25	0.16

Mean values of SGOT and SGPT value in cases and controls shows no statistically significant difference.

FIGURE 4

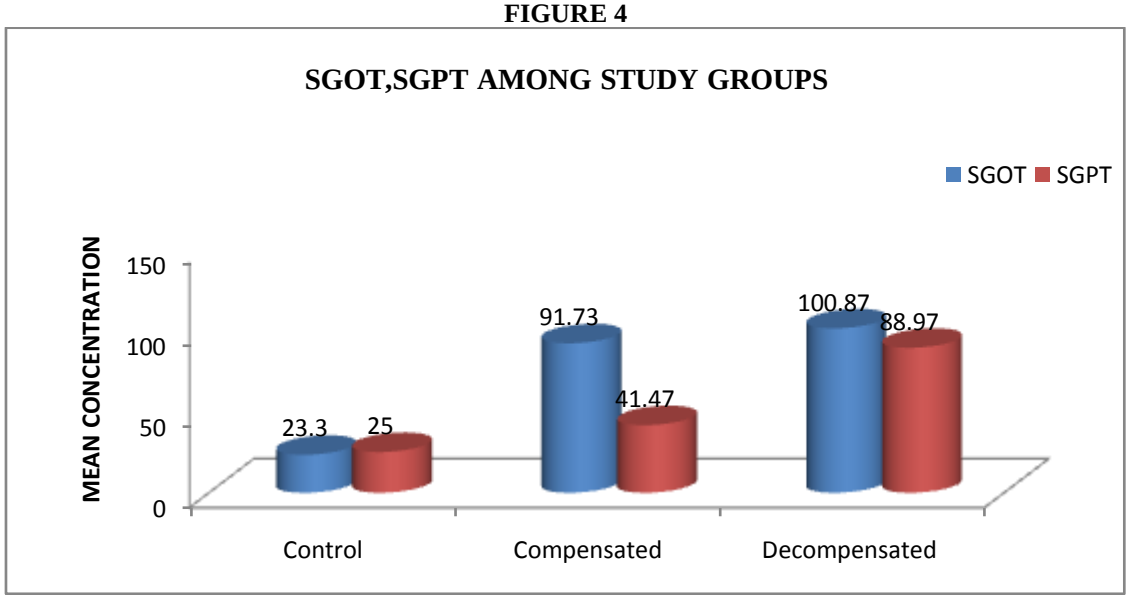

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TABLE 5
MEAN CONCENTRATION OF GGT AMONG STUDY GROUPS

	COMPENSATED	DECOMPENSATED	CONTROLS	p VALUE
GGT(U/L)	75.6	88.97	34.87	0.02

The mean value of GGT in compensated cases is 75.6, decompensated cases is 88.97 and controls is 34.87. statisticaaly significant p value<0.05 is obtained.

FIGURE 5

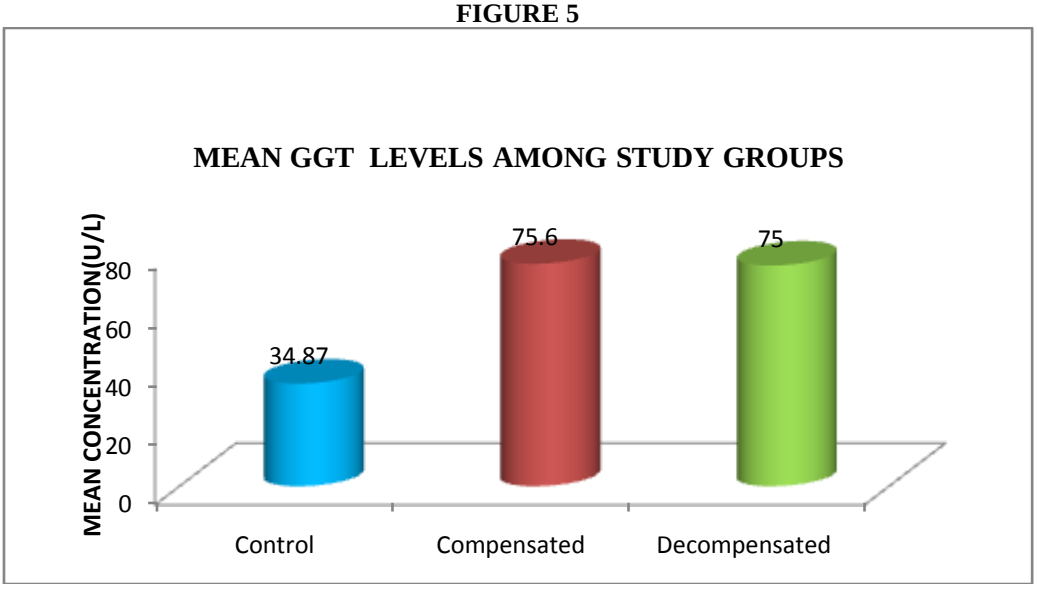

Table 6
COMPARISON OF VARIOUS PARAMETERS IN ALCOHOLIC LIVER DISEASE (COMPENSATED AND DECOMPENSATED) CASES AND HEALTHY CONTROLS

PARAMETER	**CONTROLS(n=30)**	**COMPENSATED LD(n=30)**	**DECOMPENSATED LD(n=30)**
Kallistatin(µg/mL)	24.16±3.44	14.50±3.00	12.34±3.15
Total bilirubin(mg/dL)	0.92±091	1.96±3.69	5.26±5.46
Direct bilirubin(mg/dL)	0.41±0.53	1.19±2.27	2.86±3.52
SGOT(U/L)	23.2±6.38	91.73±224.06	100.87±76.17
SGPT(U/L)	25±4.65	41.47±59.20	44.60±42.51
ALP(U/L)	72.07±11.76	84.63±46.23	137.17±16.05
GGT(U/L)	34.87±41.06	75.60±109.94	88.97±67.75
Albumin(g/dL)	4.28±0.39	3.85±0.85	2.70±0.46

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Table 6 showed (mean+ standard deviation) for all the analytes taken for study in three groups which includes healthy controls, compensated and decompensated liver disease patients. Datas are shown for parameters like total and direct bilirubin ,SGOT,SGPT , GGT, ALP, albumin and kallistatin. After comparing all parameters, this study infers that serum kallistatin levels are markedly decreased in compensated as14.50±3.0µg/mL and decompensated liver disease as 12.34+3.15µg/mL than healthy controls as 24.16±3.44µg/mL.

Table 7: ANOVA to compare the values of various biochemical parameters among three study groups.

Variable	Control			Compensated			Decompensated			p- value
	N=30			N=30			N=30			
	Mean	SD	SEM	Mean	SD	SEM	Mean	SD	SEM	
Kallistatin(µg/mL)	24.16	3.44	0.63	14.50	3.00	0.55	12.34	3.15	0.58	<0.001**-HS
Total bilirubin (mg/dL)	0.92	0.91	0.17	1.96	3.69	0.67	5.26	5.46	0.99	<0.001**-HS
Direct bilirubin (mg/dL)	0.41	0.53	0.10	1.19	2.27	0.41	2.86	3.52	0.64	0.001-S
Albumin(g/dL)	4.28	0.39	0.07	3.85	0.85	0.15	2.70	0.46	0.08	<0.001**-HS
SGOT(U/L)	23.30	6.38	1.17	91.73	224.06	40.91	100.87	76.17	13.91	0.06*-NS
SGPT(U/L)	25.00	4.65	0.85	41.47	59.20	10.81	44.60	42.51	7.76	0.16*-NS
GGT(U/L)	34.87	41.06	7.50	75.60	109.94	20.07	88.97	67.75	12.37	0.02-S

**HIGHLY SIGNIFICANT AT 1% LEVEL.
*NOT SIGNIFICANT
S-SIGNIFICANT
HS-HIGHLY SIGNIFICANT
NS-NOT SIGNIFICANT

TABLE 7 shows ANOVA to compare the values of biochemical parameters among three study groups. Statistically significant p values are obtained for kallistatin, total and direct bilirubin, albuminand GGT .Insignificant p values are obtained for SGOT and SGPT .In this study, there is a marked decrease in mean concentration of serum kallistatin levels in compensated group of 14.50±3.0µg/mL and decompensated group of 12.34±3.15µg/mL, when compared to controls as 24.16±3.4µg/mL showing a highly significant p value of <0.001.

TABLE 8
PEARSON CORRELATION (r) AND SPEARMAN RANK CORRELATION (p) BETWEEN VARIOUS PARAMETERS OF ALCOHOLIC LIVER DISEASE AND SERUM KALLISTATIN LEVELS

Parameter	Pearson correlation		Spearman rank correlation	
	r	p	r	p
Total bilirubin(mg/dL)	-0.28	<0.001**	-0.47	<0.001**

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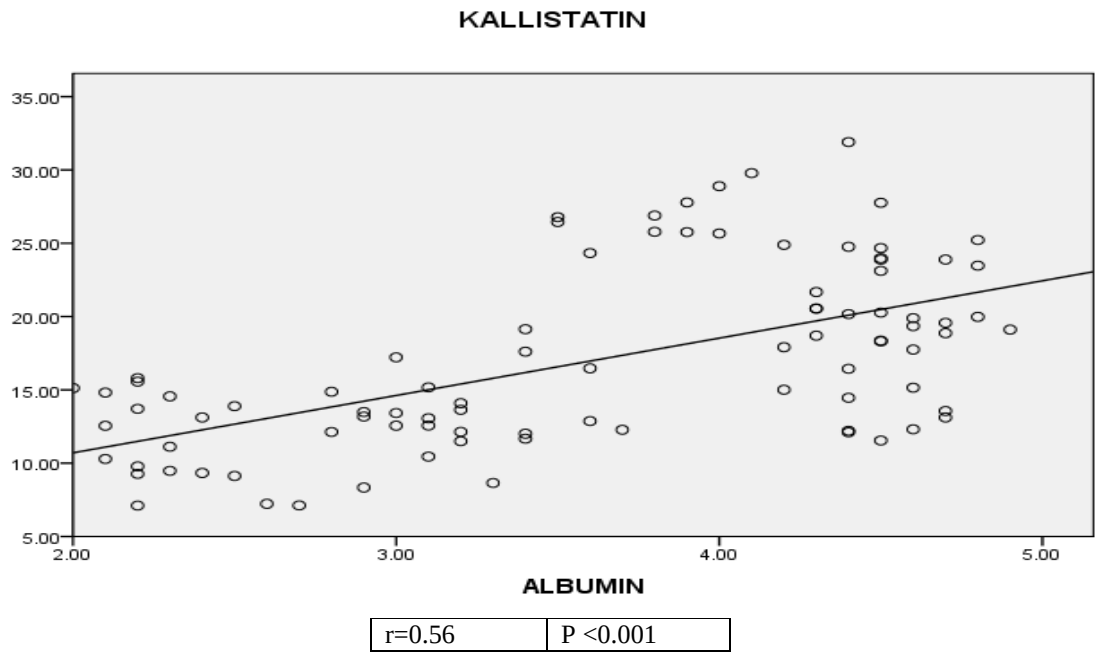
Direct bilirubin(mg/dL)	-0.29	<0.001**	-0.54	<0.001**
AST(U/L)	-0.25	0.002	-0.67	<0.001**
ALT(U/L)	-0.26	0.001		
ALP(U/L)	-0.51	<0.001**	-0.53	<0.001**
Albumin(g/dL)	0.56	<0.001**	0.55	<0.001**
GGT(U/L)	-0.24	0.003	-0.53	<0.001**

Table 8 shows Pearson and Spearman relationship between serum kallistatin levels and other liver function tests. Patients with alcoholic liver disease showed a marked positive correlation between serum kallistatin levels and serum albumin. We can also find a marked negative correlation present between serum kallistatin levels and total bilirubin, direct bilirubin, AST, ALT, ALP and GGT.

FIGURE6: CORRELATION BETWEEN SERUM KALLISTATIN LEVELS AND ALBUMIN LEVELS IN PATIENTS WITH ALCOHOLIC LIVER DISEASE



r=0.56	P <0.001

Figure 6 shows a marked positive correlation between serum kallistatin levels with an r value of 0.56 and p value of <0.001 (highly significant).

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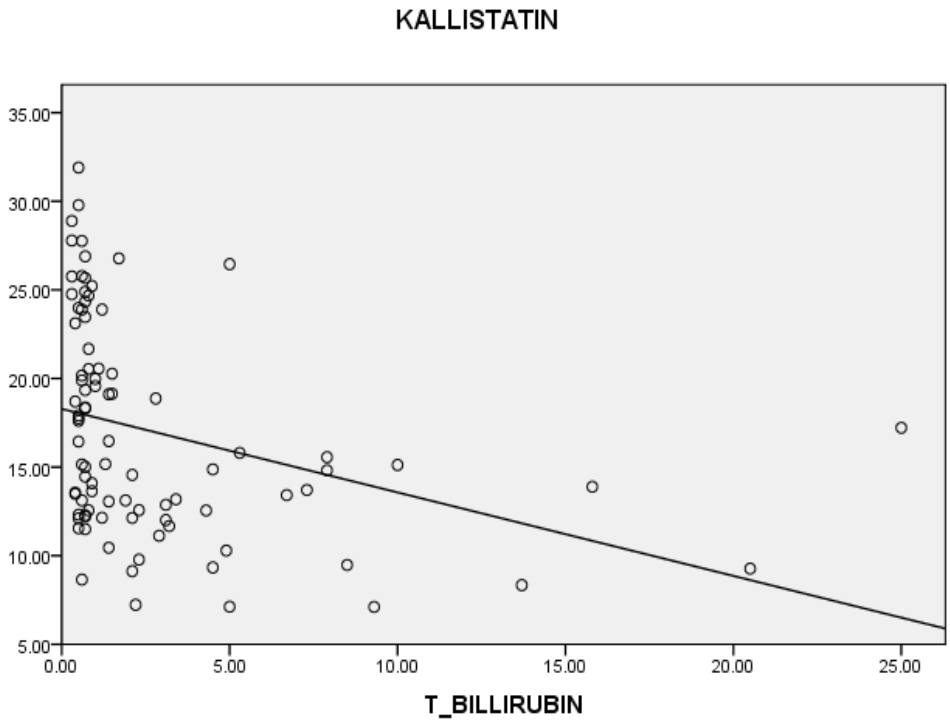
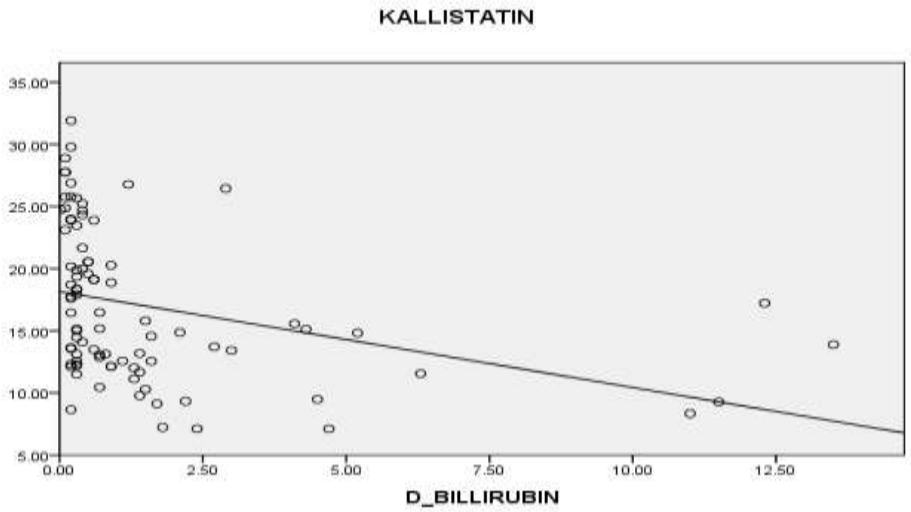
Figure 7 CORRELATION BETWEEN SERUM KALLISTATIN AND TOTAL BILIRUBIN IN PATIENTS WITH ALCOHOLIC LIVER DISEASE



r=-0.28	P< 0.001

Figure 7shows a negative correlation between serum kallistatin levels and total bilirubin levels with an r value of 0.28 and p<0.001(highly significant)

FIGURE 8 CORRELATION BETWEEN SERUM KALLISTATIN LEVELS AND DIRECT BILIRUBIN IN PATIENTS WITH ALCOHOLIC LIVER DISEASE



r=-0.29	p<0.001

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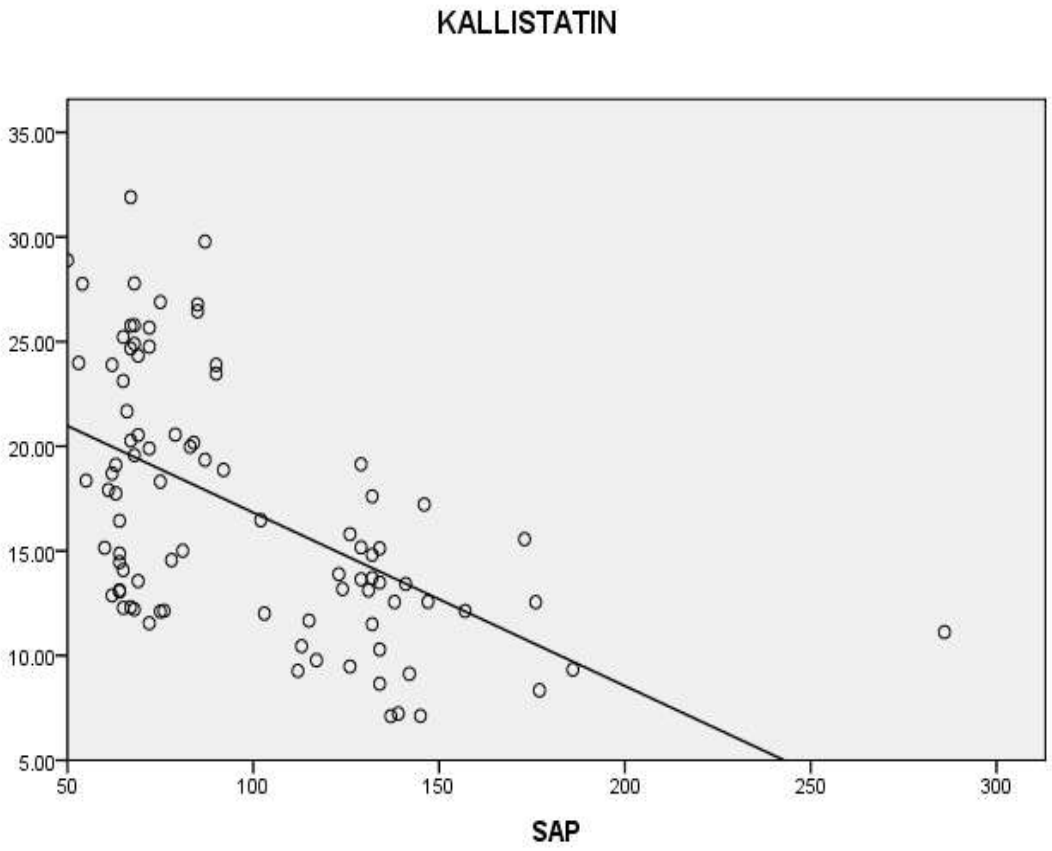
Figure 8 shows a negative correlation between serum kallistatin levels and direct billirubin levels with an r value of 0.29 and p value of <0.001(highly significant)

FIGURE 9 CORRELATION BETWEEN SERUM KALLISTATIN LEVELS AND SAP IN PATIENTS WITH ALCOHOLIC LIVER DISEASE



r=-0.51	P<0.001

Figure 9 shows a negative correlation between serum kallistatin levels and alkaline phosphatase levels with an r value of 0.51 and pvalue<0.001(highly significant)

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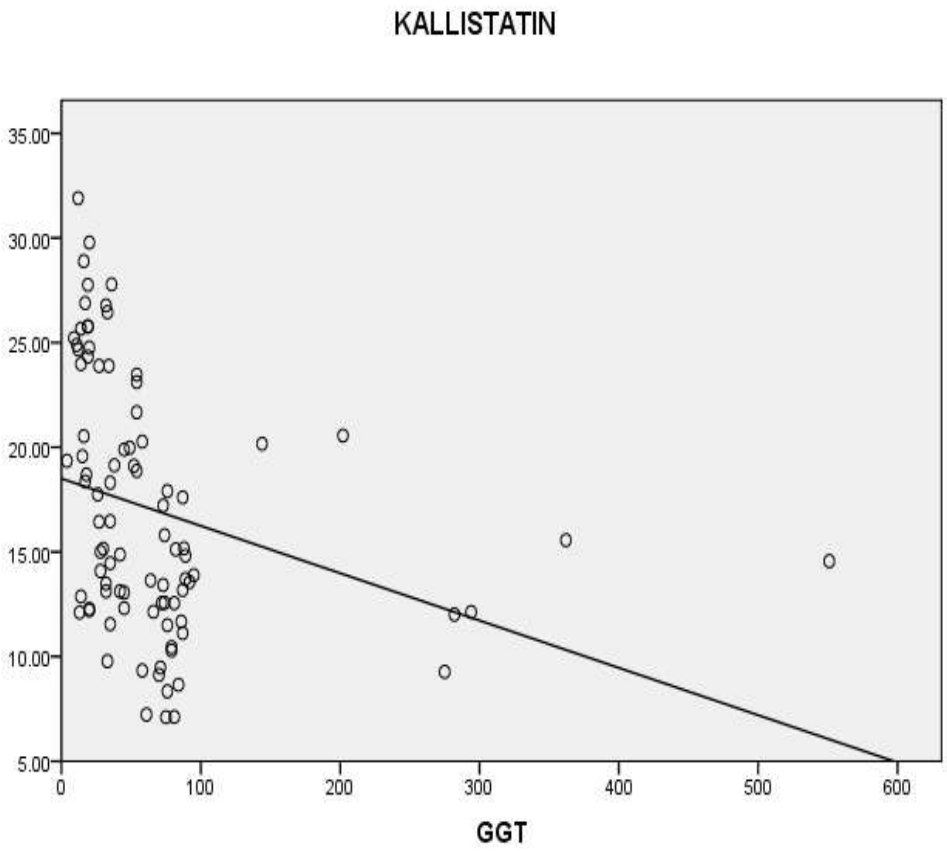
FIGURE 10 CORRELATION BETWEEN SERUM KALLISTATIN LEVELS AND GGT LEVELS IN PATIENTS WITH ALCOHOLIC LIVER DISEASE



r=0.24	P=0.003

Figure 10 shows a negative correlation between serum kallistatin levels and GGT levels with an r value of 0.24 and p value of 0.003 (significant)

TABLE 9: BASED ON ROC

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

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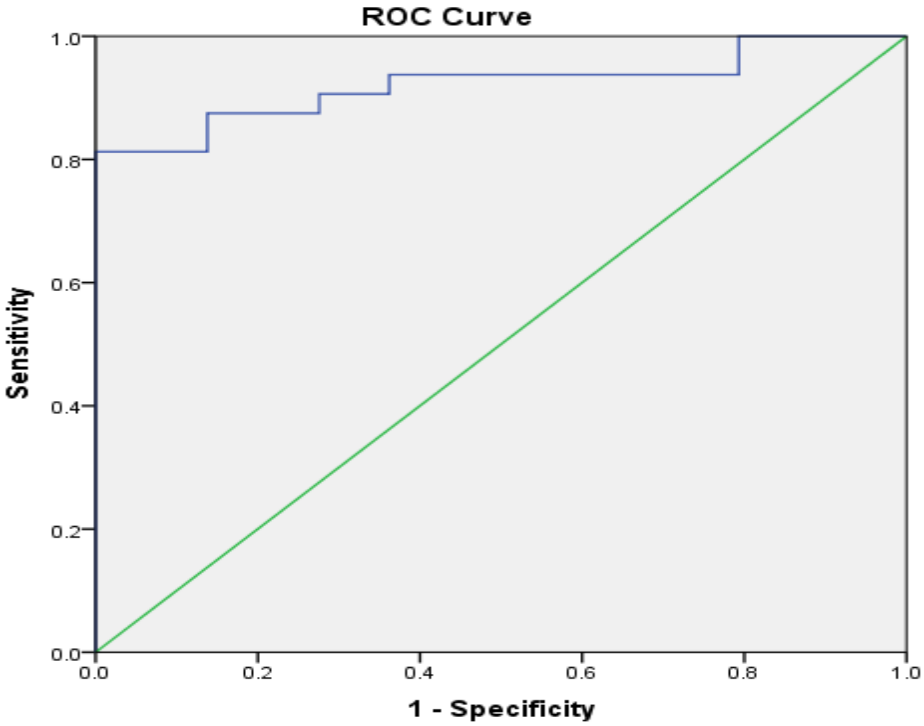

Table 9 shows a Receiver Operating Characteristic curve with x-axis showing specificity and y-axis showing sensitivity. Area under curve was 0.922 with an optimal cut off value 20.22µg/mL which proves that kallistatin can be a good non-invasive biomarker for diagnosing alcoholic liver disease. sensitivity, specificity, positive and negative predictive values are 93.65%, 96.30%, 98.33% and 86.67% respectively.

IV. DISCUSSION:

Alcoholism, a common worldwide social stigma, wherein the consumption of alcohol within saferlimits, seems to be normal. When the drinking habit becomes regular and frequent, organ damage starts. Liver is commonly affected by alcoholism. Since, the liver has a large reserve capacity, patient has no symptoms and seems to be healthy, but as the liver injury progresses, its functions are impaired obviously, indicated by a decrease in albumin synthesis[30]

In a review study, classification of liver damage on the basis of ultrasound, proved to be better than liver biopsy. Theyachieved a detection of 81.48% of compensated liver disease and 91.7% of decompensated liver disease [31,32]. Nearly 50% of patients with liver disease are identified in compensated stage itself[33].They can live for a period of ten to twelve years with compensated features. Patients with compensated features may even present with esophageal varices but once de-compensated features viz., portal hypertension, ascites and encephalopathy setin, mortality is reduced to one year. Decompensated liver disease is considered to be a precursor of hepatocellular malignancy[34].Liver performs a wide variety of functions. Functional damage occurs due to chain of disorders of other organ systems. A single test cannot identify liver damage as well as, trace out the progression of liver disease. Minor damage to liver cannot be assessed in early stage as the liver functions appear to be normal due to compensatory mechanisms. Some tests may be indicative of liver damage but they are not useful in assessing the prognosis of disease and vice versa.

Kallistatin, a non-invasive biomarker, is considered in the present study, to prove its diagnostic sensitivity in detecting liver dysfunction at an early stage and to assess the progress as it enters a vulnerable stage which causes irreversible damage to liver. In order to trace the progression of liver damage due to alcohol, with kallistatin levels, alcoholic liver disease has been categorized into compensated liver disease and de-compensated liver disease.

This study includes a control group of 30 and a study group of 60, with age limits in comparable range. The subjects of the control group as well as the study group comprised of only males since females were neither exposed with

alcoholic history nor did they present with any obvious findings in ultrasound abdomen, suggestive of alcoholic liver disease. Tradition of Indian females prevents them to come out with alcohol history in social atmosphere. This could not be considered as a limitation of study since, several studies from other countries, conveyed the essence of the findings that, kallistatin levels showed no significant difference among gender and age related groups[35,36].

After analyzing the values of all the parameters in this study, they were found to be within the reference range published in various reviews or as per the kit insert values for reference range, depending on the method adopted. Serum kallistatin levels in this study for healthy controls showed a mean concentration of 24.16+3.44 µg/mL(table 5) which is closer to the reference range of 22.1+3.5µg/mL, quoted by Chao J et al study[36].At the onset of alcoholic liver disease, sets into the decompensated stage, various analytes like total and direct bilirubin , albumin , SGOT, SGPT, ALP andGGTareaffected.kit methods are used to assay the substrates and enzymes .These methods are based on Modified IFCC procedures, enzymatic and kinetic methods .

Alcoholic liver disease patients show markedly elevated levels of conjugated bilirubin level in blood[37,38]. In this study, Figure 2 showed a markedly elevated levels of total and direct bilirubin in decompensated liver disease than compensated liver disease Synthesis of albumin decreases in cases of alcoholic liver disease often associated with malnutrition[39,40,41].

ENZYME ALTERATION IN ALCOHOLIC LIVER DISEASE:

SGOT and SGPT are present in enormous amount in hepatic cells. Whenever there is liver damage due to alcohol or toxins, this enzyme leaks out from cells and comes into circulation. Even though they act as good markers of hepatic damage, they lack sensitivity[42,49].As proposed in previous studies, Mean values of SGOT and SGPT value in cases and controls shows no statistically significant difference as shown in table 7.We can also note that aspartate transaminases levels are markedly raised when compared to alanine transaminases, as in table 7(AST level as 100.87U/L and ALT level as 44.60U/L in decompensated LD cases).This is due to the distribution of AST in enormous tissues of our body than ALT[43].Serum alkaline phosphatase is an enzyme which arises from the outer cell membrane of liver but it has entirely different activities.ALP activity is useful for finding out the etiology and extent of liver damage. In this present study, Table 6 showed serum alkaline phosphatase levels being elevated incompensated alcoholic liver disease as84.63$\pm$46.23 U/L,decompensated LD cases as137.17$\pm$16.05 U/L than healthy controls as 72.07$\pm$11. 76 U/L.Gamma glutamyl transferase is an enzyme of biliary canaliculi. Alcohol intake for a longer period of time induces hepatic damage and causes an elevation of GGT level[44,45]. In a study done by Krastev et al, it was proved that GGT levels do not elevate in the beginning of alcoholic liver disease and patient coming for follow up after treatment[46]. In Table 7, comparison of GGT levels shows statistical significance between control, compensated and decompensated liver disease with a p value<0.05 as in the study performed by S .Orlowskin et al . This increase may be due to microsomal induction and damage of hepatic cells[47,48].Literatures explored markers like AST and ALT lack either sensitivity or specificity or both. Some markers like GGT lack specificity[50,51]. This study is aimed to evaluate the role of kallistatin as a non-invasive biomarker in the diagnosis of alcoholic liver disease, its usefulness in correlation with disease severity and to compare serum kallistatin levels with apparently healthy individuals.

Correlation Between Serum Kallistatin And Other Biochemical Parameters:

Pearson and Spearman rank correlations (in table 8) done to analyse the positive and negative correlation of serum kallistatin levels and other variables included in the study. Datas showed that Serum kallistatin levels positively correlatedwith serum albumin with r=0.56 and p value<0.001 in figure 6, but negatively correlated with GGT levels with r=-0.24 and p value<0.03 in figure 10.In addition, serum kallistatin is again negatively correlated with ALP showing r=0.51 and p value<0.001 in figure 9.serum kallistatin also showed negative correlations with total bilirubin showing r=-0.28 and p value <0.001 in figure 7 and direct bilirubin with r=-0.29 and p value<0.001 in figure 8.

ANOVA study in table 7 also showed a highly significant p value of <0.001 for serum kallistatinassay .we gather from the study that albumin too, proves to be highly determining variable in relation to serum kallistatin levels with a highly significant p value of 0.001.

Receiver operating characteristic curve in table 9 also emphasises that kallistatin can be a good non-invasive biomarker in diagnosing alcoholic liver disease with a high sensitivity of 93.65% and specificity of 96.30%.positive

predictive value is 98.33% and negative predictive value is 86.67 .area under curve is 0.922 and optimal cut-off value as 20.22µg/mL.

The major pillars of the study are simultaneous measurement of most of the analytes of liver panel.Synthetic function assessed by serum albuminlevels, excretory function by serum total and direct bilirubinlevelsandenzyme profile done with AST,ALT,ALP and GGT. Evaluation of the relationship of serum kallistatin levels and other biochemical parameters are done in this study.

V. CONCLUSION:

In this study, sixty cases of alcoholic liver disease proven by ultrasound abdomen and clinical history were taken up. Age and gender matched thirty controls that showed normal ultrasound abdomen and no alcohol history was selected. Various parameters like total and direct bilirubin, albumin, SGOT, SGPT, ALPand GGT were done in sixty cases and thirty controls .In this study, we found that Serumkallistatin can be used as a parameter for identifying early damage of liver due to alcohol consumption.Serum kallistatin levels decreases as the liver damage increases .As a result, mortality due to alcoholic liver disease also increases.Serumkallistatin levels were positively correlated with albumin levels; and negatively correlated with parameters viz., total and direct bilirubin ,ALP and GGT. These results infer that, serum kallistatin levels can play a vital and protective variable in preventing alcoholic liver disease. This study leads a pathway for therapeutic intervention to be started earlier on the basis of serum kallistatinlevels. Estimation of serum kallistatin levels can be added in routine investigations for liver function tests in patients with alcoholic liver disease.

LIMITATIONS OF THE STUDY:

Small sample size, when collected from a single medical hospital, may deprive us of the exact significant values, which will be more obvious with a larger sample size, if collected from an array of hospitals.Small subgroups taken for the study hinders to put forward the relationship of some co morbidities associated with alcoholic liver disease.Follow up study could have been done, to strengthen our findings and prove the advantages of using kallistatin, as a new non-invasive biomarker, to detect liver damage and its therapeutic benefits.

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