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ESTIMATION OF LEVEL OF SERUM CHOLINESTERASE IN THE PATIENTS OF LIVER DISEASES AND NON LIVER DISEASES

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Abstract

Cholinesterase is an enzyme produced by liver, that catalyses the hydrolysis of neurotransmitter acetylcholine into choline and acetic acid. They are of two type's acetylcholine esterase known as RBC cholinesterase (true cholinesterase) and plasma cholinesterase (pseudocholinsterase). Serum cholinesterase was found to be less than normal lower limit in 94.5% patients with liver diseases in our study. So assay of this enzyme is better than conventional test like total bilirubin etc. which are raised in other diseases also. Thus serum cholinesterase can be used as important biochemical parameter to distinguish liver diseases from non-liver diseases.

KEYWORDS: Cholinesterase, Liver function test

INTRODUCTION:

Liver is the largest and one of the most important vital organs in our body. To diagnose liver diseases large number of conventional liver function tests like Bilirubin levels, Transaminase levels, Alkaline phosphatase, Total Proteins, Serum Albumin level and Albumin/Globulin ratio are being performed for last many years, yet they do not have 100% sensitivity as well as 100% specificity. Many a times conventional parameters of liver function tests are raised in non-liver diseases, like Transaminase levels in heart diseases, Alkaline phosphatase levels in bone diseases etc. So there is a need for a test which should be more specific, and sensitive for diagnosing liver diseases. As cholinesterase enzyme is produced in liver, its assay may be of importance in liver diseases. Lot of studies have been conducted in the past but requires further studies to prove its usefulness in the diagnosis of liver diseases. Present study has been planned to find out the usefulness of assay of serum cholinesterase in the diagnosis of liver diseases.

As per, Per Winkel[1] conventional liver function test are many times abnormal in illnesses which are not due to liver diseases, for example transaminase levels are raised in heart diseases, alkaline phosphatase is raised in bone diseases and bilirubin level is raised in hemolytic anemia, likewise there are many other causes of decrease in serum albumin level like

nephritic syndrome. So none of these conventional tests can individually confirm liver dysfunctions (O.O. Ogunkeye).[2]

Serum cholinesterase is produced in liver and secreted in blood stream. So estimation of serum cholinesterase reflects hepatocellular damage and is regarded as sensitive indicator of liver diseases (Turkey L.).[3] Decreased levels of serum cholinesterase reflects impaired enzyme synthesis by the liver in absence of genetic cause or known inhibitors. Decreased levels are seen in many liver diseases like liver cirrhosis, viral hepatitis, alcoholic liver disease, malignancies of liver diseases. Serial measurement of cholinesterase activity has been promoted as indicator of prognosis in patients with liver diseases and for monitoring liver functions after liver transplant.[4] So single determination of cholinesterase activity in an individual can help to distinguish liver diseases from non-liver diseases.

Present study is designed to compare usefulness of serum levels of cholinesterase with conventional liver function tests and to determine whether single estimation of serum cholinesterase can be useful as well as cost effective to distinguish liver diseases from non-liver diseases.

MATERIAL & METHODS: Present study was conducted to find usefulness of serum cholinesterase to differentiate liver diseases from non-liver diseases.

Study Area: Department of Biochemistry Jawahar Lal Nehru Medical College and Hospital, Ajmer.
Sample Size: Study comprised of 150 cases of both sexes which were divided into two groups.
Group I: Liver diseases patients – 75.
Group II: Non liver disease patients – 75.

Outcome Analysis:
Interpretation was done according to p – value. $p < 0.05$ significant.
$p < 0.001$ highly significant. $p > 0.05$ not significant.

All cases were evaluated clinically and then confirmed by sonography and were recorded on printed proforma. Conventional liver function tests and cholinesterase enzyme levels were assayed for all the patients.

Inclusion Criteria:
1. Age – All age groups.
2. Both sexes.
3. Patients having clinical and sonographic evidence of liver dysfunction.

Exclusion criteria for both groups:
1. Acute abdominal diseases
2. Chronic infectious diseases
3. Protein energy malnutrition
4. Post-operative patients
5. Organophosphorous poisoning
6. Myocardial infarction

Blood Sample Collection: Venous blood was collected from all the patients and serum was separated by centrifugation.

Commercially available ready to use reagent kits were used for estimation of various conventional liver function tests and of serum cholinesterase levels.

Sample Type: Serum (free of hemolysis).
Following investigations were done for all patients of both groups:
1. Serum cholinesterase level.

2. Total and Direct bilirubin.
3. Indirect bilirubin was calculated.
4. Alanine aminotransferase (ALT/SGPT).
5. Aspartate aminotransferase (AST/SGOT).
6. Alkaline phosphatase.
7. Total proteins.
8. Serum Albumin

Following Methods were used for Estimation of Various Enzymes:

1. Serum cholinesterase - DGKC method.[5]
2. Total and Direct bilirubin - DMSO method.[6]
3. Alanine aminotransferase - NADH, kinetic UV, method IFCC rec.[7]
4. Aspartate aminotransferase - NADH, kinetic UV method, IFCC rec.[8]
5. Total protein - Biuret method.[9]
6. Alkaline phosphatase - p-Nitrophenylphosphate, kinetic method DGKC rec.[10]
7. Serum albumin - Bromocresol Green method.[11]

STANDARD OPERATING PROCEDURE FOR CHOLINESTERASE (BUTYRYLTHIOCHOLINE KINETIC):
Name of Method: Kinetic DGKC method.
Principle of the Method: Cholinesterase hydrolyses butyrylthiocholine to butyrate and thiocholine. Thiocholine reacts with 5, 5 dithiobis – 2 nitrobenzoic acid (DTNB) to form 5 mercapto 2 nitrobenzoic acid (5 MNBA) according the following reaction.[12]

Cholinesterase
Butyrylthiocholine + H_2O ----------> Butyrate + Thiocholine
Thiocholine + DTNB ----------> 5MNBA

The rate of 5MNBA formed is measured photometrically and is proportional to the enzymatic activity of cholinesterase in the sample.

REAGENTS:

R_1 Buffer:	Phosphate pH 7.7	50 mmol/L
R_2 Substrate:	5, 5 dithiobis	
	2 nitrobeizoic acid	0.25 mmol/L
	(5, 5 DTNB)	
	Butrylcholine	7 mmol/L

PREPARATION:
Working reagent:
One tablet of R_2 substrate was dissolved in one vial of R_1. Cap &
Mix gently to dissolve content.
Stability: 2 hours at 2-8□C.

PROCEDURE:

1. Wavelength	:	405 nm
2. Cuvette	:	1 cm light path
3. Temperature	:	37□C

4. Working reagent (ml)	:	1.5
5. Sample (□L)	:	10
6. Reaction direction	:	Increasing
7. Blank	:	Distilled water
8. Delay time	:	30 sec
9. Read time	:	90 sec
10. Read Interval	:	30 sec
11. Factor (37□C)	:	43420
12. Unit	:	U/L
13. Normal low	:	4659
14. Normal high	:	14443
15. Linearity (Low) (U/L)	:	7
16. Linearity (High) (U/L)	:	9084
17. No. of readings	:	3

Mix & wait for 30 sec.
If the results obtained were greater than linearity limit, sample was diluted 1:5 with NaCl 9 g/L and multiplied by 5.
Initial reading was taken at 30 second and reading after 150 secs.

CALCULATIONS:

25□C - 30□C = □ A/30s x 22710
= U/L
37□C = □ A/30s x 43420
= U/L

REFERENCE VALUE:

25□C	**30□C**	**37□C**
3000 – 9300 U/L	3714 – 11513 U/L	4659 – 14443 U/L

STANDARD OPERATING PROCEDURE FOR BILIRUBIN TOTAL AND DIRECT (T & D BILIRUBIN):

Name of Method: DMSO, colorimetric. [13]
Similarly other test were done by colorimetric method (ready to use kits were used).

OBSERVATIONS:

LIVER DISEASES PATIENTS: Total number of cases = 75.

1. Hepatitis	31
2. Cirrhosis of liver	19
3. Obstructive jaundice	4
4. Liver abscess	10
5. Liver mass	11

Table 1: Table Showing Distribution of Patients of Liver Diseases

NON-LIVER DISEASES PATIENTS: Total number of cases = 75

1. Dermatitis	11
2. Acute respiratory infections	15
3. Cellulitis	12
4. Bone diseases	6
5. Renal failure	6
6. Anasarca	7
7. COPD	18

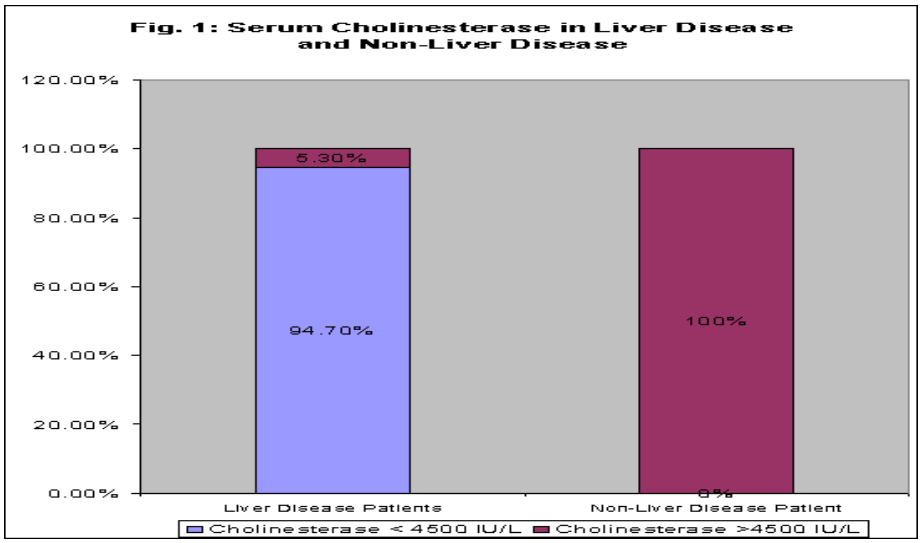
Table 1: Showing Distribution of Patients of Non-Liver Diseases

Cholinesterase (IU/L)	**Number of Liver Disease Patients**	**Number of Non-Liver Disease Patients**	**Total**
< 4500	71	0	71
>4500	04	75	79
Total	**75**	**75**	**150**

Table 2: Distribution of Patients as per Cholinesterase Levels

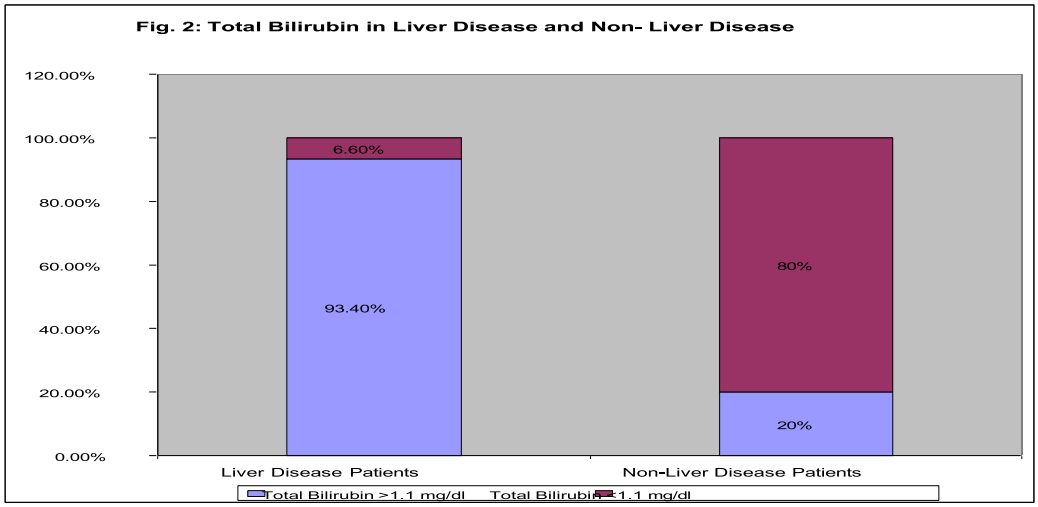
Above table shows that cholinesterase levels decreases in 94.7% cases of liver diseases but it does not decrease in non-liver disease patients. The sensitivity of serum cholinesterase to diagnose liver disease is 94.7% and the specificity is 100%.

The table below shows that total Bilirubin is raised in 93.4% cases of liver disease patients but at the same time it is also raised in 20% cases of non-liver disease patients. The sensitivity of Total Bilirubin to diagnose liver disease is 93.4%, while the specificity is 80%



Total Bilirubin (mg/dl)	Number of Liver Disease Patients	Number of Non-Liver Disease Patients	Total
>1.1	70	15	85
<1.1	5	60	65
Total	**75**	**75**	**150**

Table 3: Total Bilirubin Levels in Liver Disease and Non-Liver Disease Patients



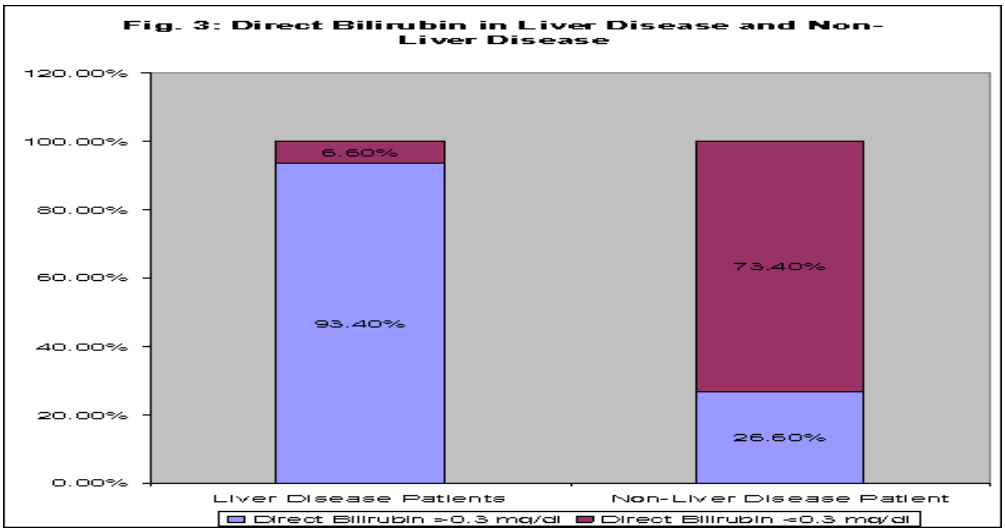
Direct Bilirubin (mg/dl)	Number of Liver Disease Patients	Number of Non-Liver Disease Patients	Total
>0.3	70	20	90

<0.3	5	55	60
Total	**75**	**75**	**150**

Table 4: Direct Bilirubin Levels in Liver Disease and Non-Liver Disease Patients

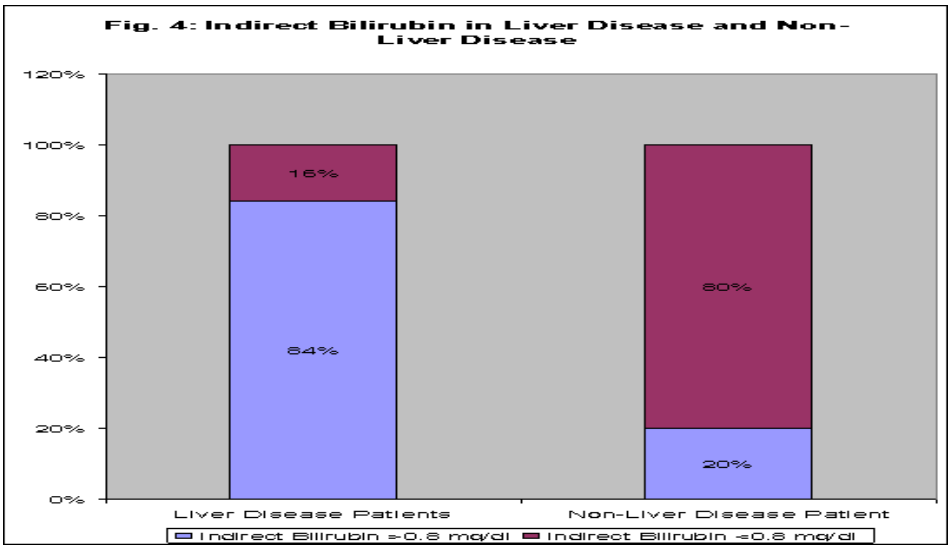
Above table shows that Direct Bilirubin is raised in 93.4% cases of liver disease patients but it is also raised in 26.6% cases of non-liver disease patients. The sensitivity of Direct Bilirubin to diagnose liver disease is 93.4%, while the specificity is 73.4%.

The table below shows that Indirect Bilirubin is raised in 84% cases of liver disease patients but also raised in 20% cases of non-liver disease patient. The sensitivity of Indirect Bilirubin to diagnose liver disease is 84%, while the specificity is 80%.



Indirect Bilirubin (mg/dl)	Number of Liver Disease Patients	Number of Non-Liver Disease Patients	Total
>0.8	63	15	78
<0.8	12	60	72
Total	**75**	**75**	**150**

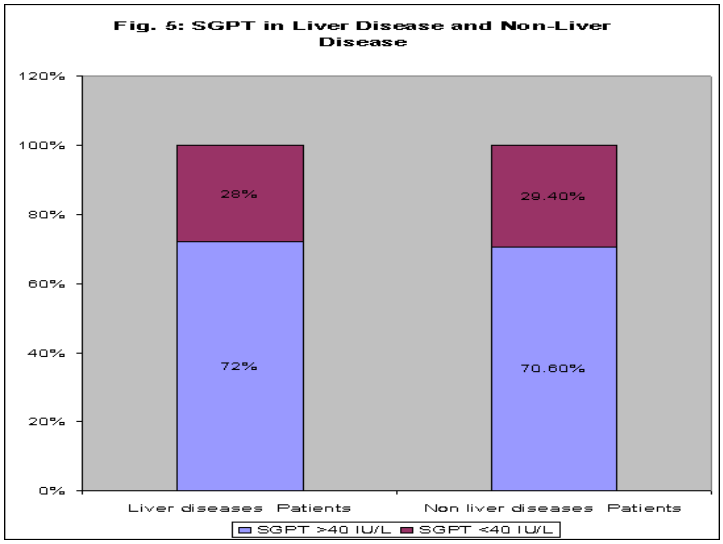
Table 5: Indirect Bilirubin Levels in Liver Disease and Non-Liver Disease Patients



SGPT (IU/L)	Number of Liver diseases Patients	Number of Non liver diseases Patients	Total
>40	54	53	107
<40	21	22	43
Total	**75**	**75**	**150**

Table 6: SGPT Levels in Liver Disease and Non-Liver Disease Patients

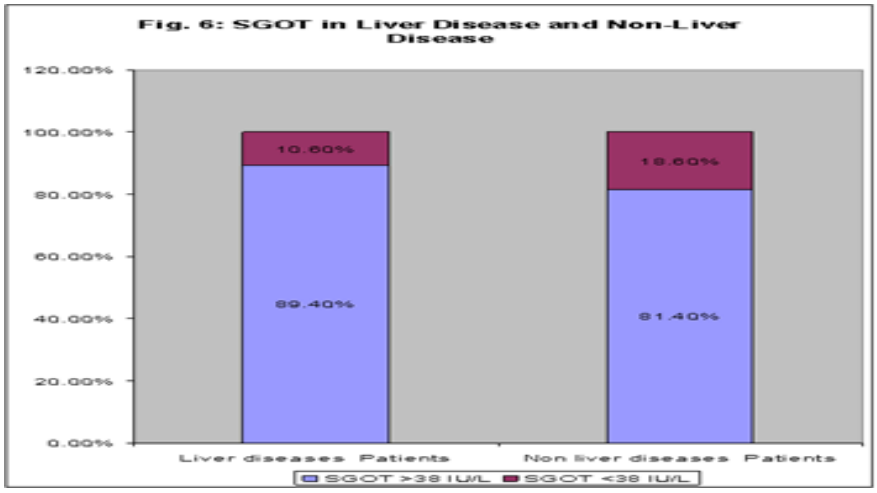
Above table shows that serum glutamate pyruvate transferase is raised not only in liver disease (72%) patients but also in non-liver disease (70.6%) patients. The sensitivity of Serum glutamate pyruvate transferase to diagnose liver disease is 72%, while the specificity is 29.4%.



SGOT (IU/L)	Number of Liver diseases Patients	Number of Non liver diseases Patients	Total
>38	67	61	128
<38	08	14	22
Total	**75**	**75**	**150**

Table 7: Serum glutamate oxaloacetate transferase Levels in Liver Disease and Non-Liver Disease Patients

Above table shows that SGOT is not only raised in liver disease patients (89.4%) but also in non-liver disease patients (81.4%). The sensitivity of SGOT to diagnose liver disease is 89.4%, while the specificity is only 18.6%.

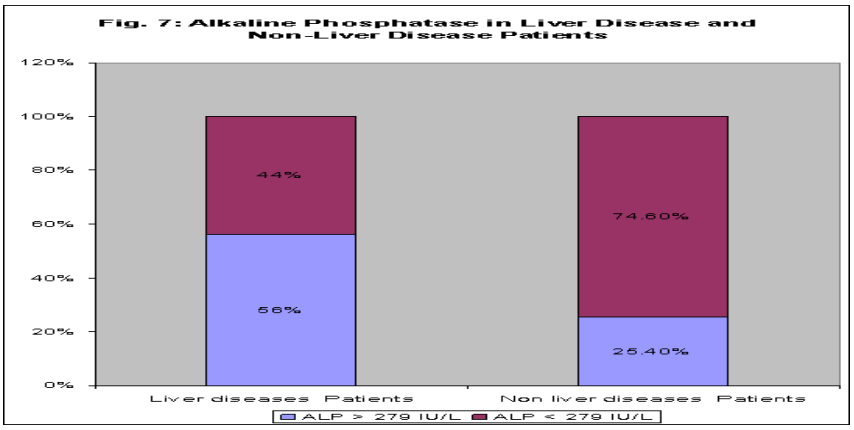


ALP (IU/L)	Number of Liver diseases Patients	Number of Non liver diseases Patients	Total
> 279	42	19	61
< 279	33	56	89
Total	75	75	150

Table 8: Alkaline Phosphatase Levels in Liver Disease and Non-Liver Disease Patients

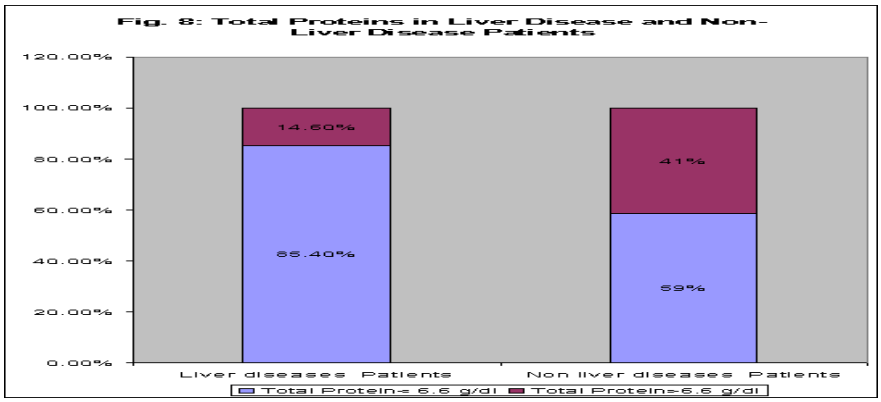
Alkaline phosphatase was raised in 56% cases of liver diseases and 25.4% non-liver diseases patients. The sensitivity of alkaline phosphatase to diagnose liver disease is 56%, while the specificity is 74.6%.

The table below shows that Total proteins are lowered in both groups of patients. 85.4% in liver disease patients while in non-liver disease patients it was 58.6%. The sensitivity of Total protein to diagnose liver disease is 85.4%, while the specificity is 41.4%.



Total Protein (g/dl)	Number of Liver diseases Patients	Number of Non liver diseases Patients	Total
< 6.6	64	44	108
>6.6	11	31	42
Total	**75**	**75**	**150**

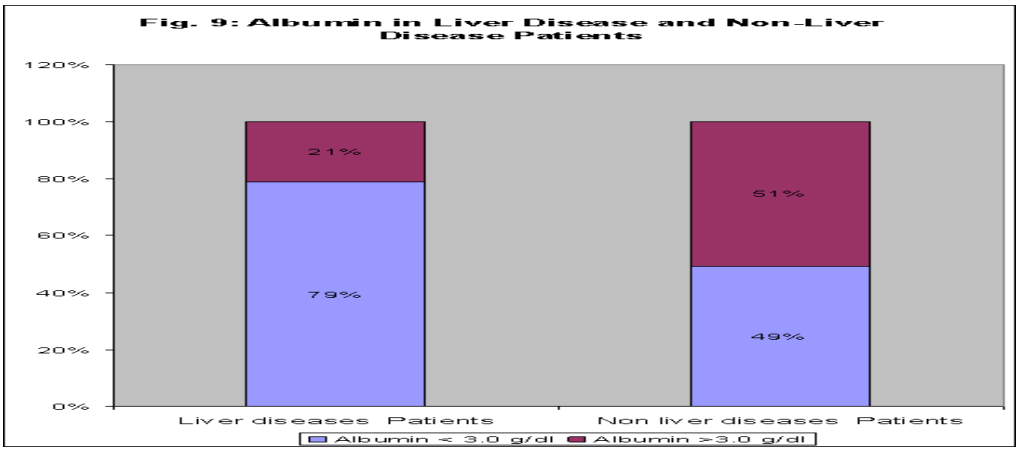
Table 9: Total Protein Levels in Liver Disease and Non-Liver Disease Patients



Albumin (g/dL)	Number of Liver diseases Patients	Number of Non liver diseases Patients	Total
< 3.0	59	37	96
>3.0	16	38	54
Total	**75**	**75**	**150**

Table 10: Serum Albumin Levels in Liver Disease and Non-Liver Disease Patients

Above table shows that Serum Albumin is not only low in liver disease (78.66%) patients but also in non-liver disease patients (49.33%). The sensitivity of Serum Albumin to diagnose liver disease is 78%, while the specificity is 50.66%.



Parameter	Biological Reference Interval	Group-1 (LD Patients) n=75			Group-2 (NLD Patients) n=75			P value
		Mini.	Maxi.	Mean±SD	Mini.	Maxi.	Mean±SD	P value
Cholinesterase (IU/L)	4659 – 14448 IU/L	831	4879	2367±900	4659	9398	6332±1316	< 0.001
Total Bilirubin (mg/dl)	Upto 1.1 mg/dl	0.3	20.6	6.4±5.5	0.4	2.0	1±0.3	< 0.05
Direct Bilirubin (mg/dl)	Upto 0.3 mg/dl	0.2	11.3	2.9±2.8	0.2	0.8	0.3±0.1	< 0.05
Indirect Bilirubin	Upto 0.8 mg/dl	0.2	11.7	3.5±3.0	0.15	1.5	0.6±0.3	< 0.05
mg/dl)								
SGPT (IU/L)	Upto 40 IU/L	13	2555	165±369	13	266	79±58	>0.05
SGOT (IU/L)	Upto 38 IU/L	15	1021	123±150	16	319	81±63	>0.05
ALP (IU/L)	98 - 279 IU/L	131	921	349±189	134	712	261±115	>0.05
Total Protein (g/dl)	6.6 - 8.3 g/dl	1.4	7.6	6.0±0.8	1.9	8.6	6.3±1.2	>0.05
Albumin (g/dl)	3.5 - 5 g/dl	1.9	4.3	3.0±0.6	1.6	4.4	3.4±0.6	>0.05

Table 11: Comparison of Serum Cholinesterase versus Conventional Liver Function Tests in Liver Disease (LD) and Non-Liver Disease (NLD)

Serum Cholinesterase levels are decreased in liver disease patients only, as compared to levels of conventional liver function tests which are decreased in both group of patients thereby showing that serum cholinesterase assay has much more significance to diagnose liver disease patients. Thus it can be stated that serum cholinesterase alone can be very helpful to distinguish liver diseases from non-liver disease.

It is observed that mean serum cholinesterase level was very much lowered in cirrhosis patients as compared to other liver diseases. SGPT and Serum glutamate oxaloacetate transferase levels were highly increased in case of hepatitis, while in cirrhosis and liver abscess patients there was minor elevations only. Alkaline phosphatase was also found to be

raised marginally. Total Proteins and Albumin were marginally decreased in all liver disease patients.

Mean activity	Cirrhosis (n=19)	Hepatitis (n=31)	Liver abscess (n=10)	Obstructive Jaundice (n=4)	Liver Mass (n=11)
Cholinesterase (IU/L)	1979	2699	2261	2654	2860
T. Bilirubin (mg/dl)	4.9	10.1	1.6	12	4.0
SGPT (IU/L)	74	350	71	26	107
SGOT (IU/L)	97	141	73	79	109
ALP (IU/L)	333	361	281	402	403
T. Protein (g/dl)	5.7	6.0	6.1	5.3	6.4
Albumin (g/dl)	2.6	2.9	3.0	2.5	3.9

Table 12: Comparison of Mean Activities of Various Parameters in Different Types of Liver Diseases

DISCUSSION:

Liver is the largest vital organ in our body. As liver has wide range of functions it is prone to many diseases which are very commonly seen in India. Conventional liver function tests have been performed for many years but many a times they are found to be abnormal in non-liver diseases also. Most of the times these tests are used in combination, but they are never effective individually to diagnose any kind of liver involvement. So, based on few abnormal tests, one can never confirm liver disease. Several studies have concluded that no single laboratory parameter is a diagnostic marker of liver dysfunction. Therefore there is a need for a test which should be specific as well as sensitive for liver diseases. As Cholinesterase enzyme is produced in the liver its assay can be of importance. Cholinesterase level may be reduced whenever there is a liver damage. Lot of research studies have been conducted but still further studies are required.

Keeping in view the findings of earlier researches the present study was conducted to find out the effectiveness of serum cholinesterase enzyme to correctly diagnose liver diseases.
Present study included patients of both sexes in the age group of 4 years to 81 years of age. Patients were evaluated clinically and confirmed ultrasonographically.

In our study majority of patients were males (58 out of 75 cases) in liver disease patients, while there were 50 in non-liver disease patients out of 75. In our study serum cholinesterase values were found to be significantly lower in liver disease patients, mean being 2367 U/L. Out of 75 cases of liver disease patients 71 cases had values less than 4500 U/L. While in non- liver disease patients all the cases had values above 4500 U/L. The difference between the mean serum cholinesterase activity of liver diseases and non-liver diseases was statistically significant. It is 94.7% sensitive and 100% specific, thereby suggesting that serum cholinesterase activity strongly indicate liver dysfunctions (Table 2, Fig. 1). Data from study conducted by M.G. Khan pointed that 100% patients with cirrhosis had lower serum cholinesterase level and he also showed that there was close relationship between the severity of cirrhosis and level of serum cholinesterase enzyme. O. Ogunkeye[14] also reported lower level of serum cholinesterase level in liver disease patients.

In the present study out of 75 patients only 5 patients of liver diseases had total bilirubin less than 1.1% mg/dl. The present study shows that estimation of serum total bilirubin might be more useful in cases of hepatitis only, but not much useful in other forms of liver diseases (Table 3, Fig. 2). In the present study 93.4% patients of liver diseases showed direct bilirubin more than 0.3mg/dl while in 26.6% of non-liver disease patients the value of direct bilirubin was more than 0.3mg/dl (Table 4, Fig.3). Indirect bilirubin in 84% cases of liver disease was found to be raised (>0.8% g/dl) but we found raised value also in 20% cases of non-liver disease patients showing specificity of 80% and sensitivity of 84% (Table 5, Fig. 4).

Over all discrepancy in levels of total, direct and indirect bilirubin levels were seen in liver diseases. In the present study, SGPT levels were raised in both group of patients i.e. 72% in liver disease patients, 70.6% in non-liver disease patients, thereby showing sensitivity of 72% and specificity of 29.4% (Table 6, Fig. 5). In the study SGOT levels were raised in both group of patients, 89.4% in liver disease patients while 81.3% in non-liver disease patients. This indicates that transaminase activity may be raised from the sources other than the liver (Table 7, Fig. 6).Our study is in accordance with the study of M.G. Khan.[15]

In present study alkaline phosphatase levels were found to be raised in 56% cases of liver disease patients and 25.4% in non-liver disease patients (Table 8, Fig. 7).Our study is similar to those of David E. Johnson, [16] B.R. Thapa, [17] Per Winkel.[1] Different isoenzyme forms of alkaline phosphatase are found in many locations in our body including osteoblasts, small intestine, proximal convoluted tubules of the kidney, placenta and white blood cells.

In the present study total protein was less than 6.6gm% in both groups of patients (95.4% in liver disease patients and 58.6% in non-liver disease patients) (Table 9, Fig. 8). Albumin constitutes major part of the total proteins while globulin contributes the second largest part of the total proteins. Albumin and globulin except immunoglobulin are produced in liver, so total protein estimation may not be useful to identify liver diseases. Albumin is decreased to <3.5g/dl in liver disease patients (78.6%) but it is decreased in non- liver disease patients (49.3%) also. Our study is similar to that of Andrew Willson[18] As per Andrew Wilson, estimation of cholinesterase provides more sensitive index of impairment of liver functions than other tests.

In our study of comparison of mean activity of serum cholinesterase in different types of liver diseases we found that cholinesterase levels were very low in cases of cirrhosis as compared to hepatitis and other liver diseases. In the present study we found that Transaminase levels were highly raised in hepatitis cases while Alkaline phosphatase levels were raised marginally. Study showed that Total protein and Albumin were marginally decreased in all liver diseases.

Thus we conclude that estimation of single parameter of serum cholinesterase is much useful in the diagnosis of liver diseases.

Mean activity U/L	**Cirrhosis n=19**	**Hepatitis n=31**	**Liver abscess n=10**	**Obstructive Jaundice n=4**	**Liver mass n=11**
Cholinesterase	1979	2699	2261	2654	2860

Comparison of mean activity of serum cholinesterase in different types of liver diseases

In the study of comparison of mean activity of various parameters in different types of non- liver diseases it is found that serum cholinesterase levels were within normal range in each group of patients while other parameters of conventional liver function test were fluctuating in different group of patients.

SUMMARY & CONCLUSION:

In our study serum cholinesterase levels were found to be significantly decreased (< 4500U/L) in majority of cases of liver disease. Cholinesterase level decreased in 94.7% cases of liver diseases but it does not decrease in non-liver disease patients. Total bilirubin is raised in 93.4% cases of liver disease patients but at the same time it is also raised in 20% cases of non-liver disease patients. Direct bilirubin is raised in 93.4% cases of liver disease patients but it is also raised in 26.6% cases of non-liver disease patients. Indirect bilirubin is raised in 84% cases of liver disease patients but also raised in 20% cases of non-liver disease patients. SGPT is raised not only in liver diseases (72%) but also in non-liver diseases (70.6%). SGOT is raised not only in liver disease patients (89.4%) but also in non-liver disease patients (81.3%). Alkaline phosphatase was raised in 56% cases of liver diseases and 25.4% non-liver disease patients.

Total protein levels were lowered in both groups of patients 85.3% in liver disease patients while in non-liver diseases patients it was 58.6%. Serum albumin was not only low in liver disease (79%) patients but also in non-liver disease patients (49%).

In the study of comparison of serum cholinesterase levels versus conventional liver function tests in both groups, it is found that serum cholinesterase levels were decreased only in liver disease patients but conventional liver function tests were abnormal in both groups of patients. Our study clearly showed that assay of cholinesterase level had sensitivity of 94.7% and specificity of 100% to diagnose liver dysfunction.

In the study of comparison of mean levels of various parameters in different types of liver diseases it is found that serum cholinesterase levels were very much low in cirrhosis liver patients as compared to other liver diseases, transaminase levels were found to be highly increased in hepatitis cases. Alkaline phosphatase levels were also found to be raised marginally; total proteins and albumin were found to be low marginally.

It is concluded that no single parameters of conventional liver function tests had effective sensitivity and specificity while serum cholinesterase had 94.7% sensitivity and 100% specificity to diagnose liver diseases thus serum cholinesterase alone can distinguish liver diseases from non-liver diseases.

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