"Estimation of 5' Nucleotidase and Serum Cholinesterase as Diagnostic Marker to Distinguish Between Various Liver Diseases and Non Liver Diseases"

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Abstract
Background*: The present study was undertaken to determine the diagnostic importance of 5'nucleotidase, serum cholinesterase enzyme activity in the patients of liver diseases and also to study the levels of different enzymes like alanine transaminase, aspartate transaminase, total bilirubin, serum albumin , and alkaline phosphatise in different liver diseases(liver cirrhosis, hepatitis, liver abscess, obstructive jaundice).*
Method: *The present study was conducted on 150 subjects, of either sex of various age groups. Study included liver disorder patients (75) attending Medical OPD, patients admitted in medical and surgical wards and patients coming in clinical laboratory, Biochemistry Department J.L.N. Medical College & Hospital, Ajmer. The results of patients were compared with the hundred non liver disease patients of either sex of similar age group. The indices included :- 5'nucleotidase (by colorimetric method of cambell), serum cholinesterase (by DGKC, colorimetric method) alanine transaminase, aspartate transaminase (by NADH kinetic UV method), total bilirubin (by DMSO method), serum albumin (by bromocresol geen method), and alkaline phosphatise (p- nitrophenyl phosphate, kinetic method).*
Result*- The mean serum 5'Nucleotidase levels, were significantly raise in liver diseases patients in comparison to non liver disease patients ($p<0.001$) (HS). Mean serum cholinesterase levels were low in in liver disease patients <1979 IU/l comparison to non liver disease patients. Total bilirubin level was raised in 93.7% liver disease patients, Aspartate transaminase was raised in 84.9% patients but also in non liver disease patients (81.4%). Similarly Alanine tranaminase, Total protein and serum albumin level were low in both liver disease patients and non liver disease patients Alkaline phosphatise was raised in both in liver disease and non liver disease patients, so, it was concluded in that serum cholinesterase and 5' nucleotidase are better diagnostic marker then the conventional liver function test that are are raised in other liver disease also.*

Keywords*: 5' Nucleotidase, Serum cholinesterase, Liver diseases.*

I. INTRODUCTION

Liver is the largest and one of the most vital organ in our body .To diagnose liver disease large number of conventional liver function test like Total bilrubin (direct and indirect), serum transaminase level, alkaline phosphotase 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 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. 5' nucleotidase is an intrinsic membrane glycoprotein present in wide variety of mammalian cell[1} Dixon and purdon first observed the clinical usefulness of 5'nucleotidase for differential diagnosis of hepatobilliary diseases and osseous diseases.The increased activity of 5' nucleotidase in liver diseases was reported {2}similar data has been reported by various author , Goldberg et al. 1973 , Rechling et al. 1988{3,4}. Lot of studies have been conducted in the past but requires further studies to prove usefulness of these diagnostic marker in the diagnosis of liver diseases. The increased level of 5' nucleotidase was seen in liver diseases .About 2.5 folds of 5' nucleotidase was seen in the liver diseases ,the probable cause proposed by Mohd.et al. (2016) are that when bile stasis occurs , biliary glycosidase cleaves the glycosyl –phosphotidylinositol moiety that anchors ecto -5'NT to the plasma membrane of bile canalicular cells and peri-portal hepatocyte and second reason given was detergent action of bile salts may then enable the liberate 5'NT to enter the circulation.(F. William et al. 1990).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}

Present study was planned to find out the usefulness of assay of 5' nucleotidase and serum cholinesterase in the diagnosis of liver diseases.

II. MATERIAL AND METHODS

A. *Study Area*:

Department of biochemistry Jawahar Lal Nehru Medical College And Hospital

B. *Sample Size*:

Study comprised of 150 cases of both sexes which were divided into two groups

GROUP I : Liver disease patients 75

GROUP II : Non liver disease patients -75

STUDY DESIGN :Descriptive comparative study

C. *Blood Sample Collection*:

Venous blood was collected from all the patients and serum was separated by centrifugation . Serum sample was free of hemolysis .

1) *Statistical Analysis*

Statistical analysis are expressed (Table-& Figures) as Mean ±SEM. Statistical analysis was performed by using Statistical Package for the Social Sciences (SPSS, version 16) and Microsoft excel. T test was performed and P values were calculated for the biochemical parameters. P values less than 0.05 were considered statistically significant.

2) *Results were Analysed by Following Methods*

Serum 5'nucleotidase was anaysed by 5' NT Method of Campbell colorimetric method , serum cholinesterase (by DGKC , colorimetric method) alanine transaminase , aspartate transaminase (by NADH kinetic UV method), total bilirubin (by DMSO method) ,serum albumin (by bromocresol geen method), and alkaline phosphatase (p-nitrophenylphosphate , kinetic method).

3) *Ethical Consideration*

Ethical approval was obtained and the institutional guidelines were followed

Observation	
Hepatitis	31
Cirrhosis of liver	19
Obstructive jaundice	4
Liver abscess	10
Liver mass	11

Table 1: Table Showing Distribution of Patients of Liver Diseases (n= 75)

Parameter	Biological Reference Interval	Group I LD patients (N=75) Mean±S.D.	Group II Non-Liver disease patients (N=75) Mean±S.D.	P value
5' Nucleotidase (IU/L)	2-10	24±1.0	11.0±3.42	< 0.001
Cholinesterase (IU/L	4659-14448 (IU/L)	2367±900	6332±1316	< 0.001
Total Bilirubin (mg/dl)	Upto 1.1 (mg/dl)	6.4±5.5	1±0.3	< 0.05
Direct Bilirubin (mg/dl)	Upto 0.3 (mg/dl)	2.9±2.8	0.3±0.1	< 0.05
Indirect Bilirubin (mg/dl)	Upto 0.8 (mg/dl)	3.5±3.0	0.6±0.3	< 0.05
SGPT (IU/L)	Upto 40 (IU/L) 165±369	165±369	79±58	> 0.05
SGOT (IU/L)	Upto 38 (IU/L)	123±150	81±63	> 0.05
ALP (IU/L)	98 - 279 (IU/L)	349±189	261±115	> 0.05
Total Protein (g/dl)	6.6-8.3 (g/dl)	6.0±1.2	6.3±1.2	> 0.05
Albumin (g/dl)	3.5 (g/dl)	3.0±0.6	3.4±0.6	> 0.05

Table 2: Comparison Of 5' Nucleotidase And Serum Cholinesterase Versus Conventional Liver Function Test
(N = Total Number Patients, LD = Liver Disease Patients)

Mean activity	Cirrhosis N =19	Hepatitis N =31	Liver abscess N=10	Obstructive Jaundice N=4	Liver Mass N=11
5' Nucleotidase	24. 87±3.42	35.2±8.43	17.2±3.42	27.03±11.59	18.7±5.42
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 3: Comparison of Mean Activities of Various Parameters in Different Types of Liver Diseases

III. RESULTS

5' nucleotidase levels were increased in liver disease patients only, the mean value was found to be 24±3.42 (table 2), as compared to non liver disease patients , and serum cholinesterase level was decreased in liver disease patients the mean value found was 2367 ± 900 IU/ L in comparison to non liver disease patients which was 6332(Table 2) .Total bilrubin mean value in the liver disease patients was found to be 6.4±5.5 mg / dl , Direct bilirubin 2.9 ± 2,8, Indirect Bilirubin 3.5±3.0,SGPT 165± 165 ± 367 , Alkaline phosphatase 349±189IU/l, Total protein 6.0±0.8 and serum albumin 3.0±0.6 g/dl. So it was

clearly seen that 5'nucleotidase and serum cholinesterase is of great diagnostic importance in liver disease patient in comparison to the conventional liver function test.

Serum 5'-NT levels were significantly raised in cirrhosis liver, hepatitis , liver abscess , obstructive jaundice , liver mass. The levels were 24.87±8.43, 35.2±43, 17±3.42, 27±11.59, 18.7±5.42 respectively when compared to non liver disease patients (11.2±3.42) IU/L.SGPT and serum glutamate oxaloacetate tranferase levels were highly increased in case of hepatitis while in cirrhosis, and liver abscess patients there was minor elevation. Alkaline phospahatase was found to be raised marginally . Total Protein and albumin level were markedly decreased in all the liver disease patients (table 3).

IV. DISCUSSION

Liver is the largest vital organ in our body, serves many vital functions such as remove damaged red blood cells from the blood in co-ordination with spleen, produces bile, clotting factors, stores vitamins, minerals, protein, fats and glucose from diet [17]. Disease of liver and biliary tract always calls for determination of liver enzymes as opposed to other parameters . Since the liver performs a variety of functions so no single test is sufficient to provide complete estimate of liver damage (Mohd. et al .2016).Mean value of 5' Nucleotidase was found to be 24.±10 in liver disease patients which was found to statically significant p<0,001. In comparison to the non liver disease patients 11.0±3.42 . Similar results were shown by Mohd . et al 2016 where in his study he concluded that Serum 5'-nucleotidase levels showed elevations in mean value of three fold in viral hepatitis, 2.5 folds elevation in alcoholic liver disease and two fold in cirrhosis as compared of healthy control subjects.

In our study serum cholinesterase values were found to be significantly low in liver disease patients, in cirrhosis the value was as low as 1979 IU/l . The mean value being 2367IU/l .

Out of 75 cases of liver disease patients 71 cases had the values less than 4500IU/ L, while in non liver disease patients all the cases had values above 4500IU/l. The values were found to be 94.7% sensitive and 100%specific, thereby suggesting that serum cholinesterase activity strongly indicates liver dysfunction.

Data from the 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 the level of serum cholinesterase

O. Ogunkeye et al.(2010) also reported lowered level of serum cholinesterase level in 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%).

So it is clearly seen in the present study that 5'nucleotidase is significantly increases in liver disease patients (table 2,3), and when intercomparision was done between the different liver disease patients level was found to be highest in hepatitis patients (table 3). When the levels were compared to alkaline phosphatase the value was found to be statistically significant and had a positive correlation with alkaline phosphatase. Similar results was also shown by Anil et al. (2011), it was concluded his study that the variation of alkaline phosphatase and 5'NT frequently run parallel to each other in patients with liver disease probably reflects there identical location in liver . but 5' NT did not increased in bone disease

It was also seen that when , 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.(table 3)

V. CONCLUSION

It is concluded that no single parameters of conventional liver function tests had effective sensitivity and specificity while 5' nucleotidase and serum cholinesterase had more sensitivity and specificity to diagnose liver diseases thus 5' nucleotidase and serum cholinesterase can distinguish liver diseases from non-liver diseases.

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