Serum cholinesterase as diagnostic marker of liver disease

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Abstract
Background: Liver disease is leading cause of morbidity and mortality worldwide. Cholinesterase is a family of enzymes that catalyse the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid. It is an enzyme synthesized by hepatocytes and its serum levels reflect the synthetic function of liver.

Objectives: To estimate serum cholinesterase in liver disease patients and to compare serum cholinesterase level with other liver function tests like SGOT, SGPT, ALP and Bilirubin levels.

Methodology: The present cross sectional study was conducted at the Biochemistry department of tertiary care institute. Thirty patients with liver disease were included in the group A and 30 healthy patients not having liver disease were enrolled in group B as control. Serum cholinesterase and Liver Function Tests were estimated in all participants.

Results: The levels of cholinesterase were significantly lower in liver disease patients. Serum cholinesterase was 3424.77 ± 2149.30 in group A vs. 7320.77 ± 1577.26 in group B (P<0.05). It is 90% sensitive and 100% specific.

Conclusion: From the present study it is concluded that serum cholinesterase can serve as better diagnostic marker of liver disease.

Keywords: Serum cholinesterase, Liver Function Test, Sensitivity.

1. Introduction
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. Liver disease is any disturbance of liver function that causes illness. It is also referred to as hepatic disease. It is major leading cause of morbidity and mortality worldwide. Biochemical tests for the assessment of liver function (commonly referred to as liver function tests) includes measurement of serum aspartate and alanine transaminases, serum bilirubin, serum albumin, serum protein and alkaline phosphatase but these tests are often abnormal in patients with clinical problems other than liver dysfunction. The activities of serum transaminases may be raised due to increased release from non-liver tissue sources in various pathologies. Increased serum alkaline phosphatase activity may result from physiological or pathological enzyme production and release from non-liver tissue sources. Serum bilirubin may be raised because of increased erythrocyte breakdown rather than because of failure of hepatic clearance. Albumin concentration may be reduced for reasons other than failure of liver synthesis. As a result, none of these tests can individually confirm liver dysfunction. Therefore there is a need for a test which should be specific as well as sensitive for liver diseases. Cholinesterase is a family of enzymes that catalyse the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid. The 2 types of cholinesterase found in the human blood are acetylcholinesterase ("true" cholinesterase) in red cells and butyryl cholinesterase (non-specific, pseudo cholinesterase) in serum.

Cholinesterase (ChE) is synthesized mainly in hepatocytes and released into the blood. Serum ChE activity is reduced in liver dysfunction due to reduced synthesis. The predominant hepatic source of serum cholinesterase, the marked decrease in its synthesis with hepatocyte dysfunction and restoration of synthesis with hepatocyte recovery suggests that serum cholinesterase activity might be a more specific indicator of liver dysfunction than the traditional liver function tests.

The present study was conducted to estimate serum cholinesterase in liver disease and to compare it with other liver function tests.

2. Material and Methods
2.1 Study setting & study type
The present cross sectional study was conducted at the Biochemistry department of Dhiraj Hospital, Piparia, Vadodara after approval from institutional ethical committee.

2.2 Study participants & study period
During August 2014 patients having liver diseases in the age group of 20-70 years of either sex attending the OPD or admitted in ward were included in Group A or case group and Healthy subjects without liver disease were enrolled in Group B or control group.

2.3 Inclusion criteria
Patients with any liver disease (any four of SGPT, SGOT, ALP, Bilirubin, albumin were abnormal) like hepatitis, liver cirrhosis, jaundice, liver abscess were included in this study.

2.4 Exclusion criteria
The patients with age <20 or >70 years, having acute infection, chronic malnutrition, poisoning from organophosphates were excluded. Females having pregnancy or using oral contraceptive pills were excluded from the study.

2.5 Sample size and sampling
Purposively 30 patients with liver disease were included in the group A and 30 healthy patients not having liver disease were enrolled in group B.
2.6 Data collection

Written consent was taken from all participants before entering in the study. First brief socio demographic information from all participants was collected. Blood samples of participants were taken from cubital vein and collected in plain tubes. Serum Cholinesterase (reference range: 4850-12000 U/L) was estimated by enzymatic method on Erba semi auto analyzer. Total bilirubin (reference range: 0.2 – 1.0 mg/dl) and direct bilirubin (reference range: 0.1 – 0.4 mg/dl) were estimated by diazo method. Total Protein (reference range: 6 – 8 gm/dl) was measured by Biuret method and Albumin (reference range: 2.7 – 5 gm/dl) by BCG method on Erba EM-200 fully automated analyzer. Serum Glutamic Oxaloacetic Transaminase (SGOT) (reference range: up to 40 U/L), Serum Glutamate-Pyruvate Transaminase (SGPT) (reference range: up to 40 U/L) and Alkaline Phosphatase (ALP) (reference range: Adult: 80 – 290 U/L, Child: 245 – 770 U/L) were estimated by enzymatic method on Erba EM-200 fully automated analyzer.

3. Statistical Analysis

The data were entered in Microsoft excel 2007. Statistical analysis was done by Epi info 7. Continuous variables were expressed as mean ± Standard Deviation and categorical variables were expressed as percentages. Statistical analysis of different biochemical parameters was performed by Students’ t-test. Chi square or fisher’s test were used for categorical analysis accordingly. A value of p< 0.05 was considered as statistically significant.

4. Results

The mean age in both the groups was 50.50 ± 12.54. Out of 30 subjects in group A 24 (80%) were male and 6 (20%) were female, in group B 25 (83.3%) were male and 5 (16.7%) were female. Out of 30, 14 (46.7%) were taking alcohol in group A and 8 (26.7%) in group B. Serum cholinesterase level were found to be low in 27 (90%) patients in group A and normal in 3 (10%) patients while in group B 25 (83.3%) were male and 5 (16.7%) were female. Out of 30, 14 (46.7%) were taking alcohol in group A and 8 (26.7%) in group B. Smoking was considered as 16 (53.3%) in group A and 9 (30%) in group B. Total Protein (reference range: 5.29 ± 1.09 g/dl) was estimated by enzymatic method. Total bilirubin (reference range: Child: 8.44 ± 0.81 mg/dl, Adult: 12.9 ± 0.9 mg/dl) was estimated by diazo method. Total Protein (reference range: 5.29 ± 1.09 g/dl) was estimated by Biuret method and Albumin (reference range: 2.7 – 5 gm/dl) by BCG method on Erba EM-200 fully automated analyzer. The difference between the mean serum cholinesterase activity of liver diseases and control group was statistically significant with 90% sensitivity and 100% specificity, suggesting that reduced serum cholinesterase activity strongly indicate liver dysfunctions.

Table 1: Socio demographic information of the both groups

| Characteristics of participants | Group A-Cases (n=30) | Group B- Control (n=30) |
|---------------------------------|----------------------|------------------------|
| Age distribution               |                      |                        |
| 20-30 years                     | 2 (6.7%)             | 2 (6.7%)               |
| 31-40 years                     | 4 (13.3%)            | 4 (13.3%)              |
| 41-50 years                     | 7 (23.3%)            | 7 (23.3%)              |
| 51-60 years                     | 10 (33.3%)           | 10 (33.3%)             |
| 61-70 years                     | 7 (23.3%)            | 7 (23.3%)              |
| Sex                             |                      |                        |
| Male                            | 24 (80%)             | 25 (83.3%)             |
| Female                          | 6 (20%)              | 5 (16.7%)              |
| Literacy                        |                      |                        |
| Literate                        | 22 (73.3%)           | 24 (80%)               |
| Illiterate                      | 8 (26.7%)            | 6 (20%)                |
| Marital status                  |                      |                        |
| Married                         | 28 (93.3%)           | 29 (96.7%)             |
| Unmarried/single/divorced       | 2 (6.7%)             | 1 (3.3%)               |
| Smoking                         |                      |                        |
| Yes                             | 11 (36.7%)           | 9 (30%)                |
| No                              | 19 (63.3%)           | 21 (70%)               |
| Drinking alcohol                |                      |                        |
| Yes                             | 14 (46.7%)           | 8 (26.7%)              |
| No                              | 16 (53.3%)           | 22 (73.3%)             |
| Serum cholinesterase level      |                      |                        |
| Low                             | 27 (90%)             | 0 (0%)                 |
| Normal                          | 3 (10%)              | 30 (100%)              |
| High                            | 0 (0%)               | 0 (0%)                 |

In the present study the level of cholinesterase were significantly lower in liver disease patients, mean being 3424.77 U/L as compared to controls. (Table 2). Total bilirubin, direct bilirubin and indirect bilirubin were significantly higher in Group A as compared to Group B. SGPT, SGOT and ALP were also significantly higher in patients with liver disease as compared to control (Table 2). Serum total protein and serum albumin were significantly lower in cases compared to controls. (Table -2)

Table 2: Comparison of Means of laboratory parameters in both groups

| Variables                  | Group A-Cases (n=30) | Group B- Control (n=30) | P value |
|----------------------------|----------------------|-------------------------|---------|
| Serum cholinesterase       | 3424.77 ± 2149.30    | 7320.77 ± 1577.26       | <0.05   |
| Total bilirubin            | 4.33 ± 3.40          | 0.78 ± 0.50             | <0.05   |
| Direct bilirubin           | 2.82 ± 2.57          | 0.40 ± 0.38             | <0.05   |
| Indirect bilirubin         | 1.50 ± 1.17          | 0.39 ± 0.24             | <0.05   |
| SGPT                       | 99.96 ± 493.33       | 37.73 ± 35.15           | 0.002   |
| SGOT                       | 88.90 ± 76.36        | 43.30 ± 34.58           | 0.005   |
| ALP                        | 313.20 ± 97.15       | 206.13 ± 73.06          | <0.05   |
| Total protein              | 5.29 ± 1.09          | 6.22 ± 0.98             | 0.001   |
| Serum albumin              | 2.66 ± 0.79          | 3.46 ± 0.81             | <0.05   |

(SGOT - Serum Glutamic Oxaloacetic Transaminase, SGPT - Serum Glutamate Pyruvate Transaminase, ALP - Alkaline Phosphatase)

Out of 30 cases of liver disease patients 27 cases had cholinesterase values less than 4850 U/L. While in control group all the participants had values above 4850 U/L. The difference between the mean serum cholinesterase activity of liver diseases and control group was statistically significant with 90% sensitivity and 100% specificity, suggesting that reduced serum cholinesterase activity strongly indicate liver dysfunctions.
Out of 30 cases of liver disease patients 25(83.33%) cases had bilirubin values more than 1.0 mg/dl. While in control group 5 (16.66%) had bilirubin values more than 1.0 mg/dl, showing sensitivity and specificity of 83.33%. (Table-3)

### Table 3: Individual sensitivity and specificity of Biochemical markers of Liver disease

| Variables                  | Liver disease (n=30) | No liver disease (n=30) | Sensitivity | Specificity | p-value |
|----------------------------|----------------------|-------------------------|-------------|-------------|---------|
| Serum cholinesterase       | <4850                | ≥4850                   | 27          | 00          | 90%     | 100%    | <0.001  |
| Total bilirubin            | > 1.0 g/dl           | ≤1.0 g/dl               | 25          | 05          | 83.33%  | 83.33%  | <0.001  |
| SGPT                       | >40 U/L              | ≤40 U/L                 | 24          | 04          | 80%     | 86.67%  | <0.001  |
| SGOT                       | >40 U/L              | ≤40 U/L                 | 22          | 10          | 73.33%  | 66.67%  | 0.004   |
| ALP                        | >290 U/L             | ≤290 U/L                | 18          | 05          | 60%     | 83.33%  | 0.001   |
| Total protein              | >6.0 g/dl            | ≥6.0 g/dl               | 23          | 12          | 76.67%  | 60%     | 0.008   |
| Serum albumin              | <2.7 gm/dl           | ≥2.7 gm/dl              | 20          | 05          | 66.67%  | 83.33%  | 0.0002  |

(SGOT - Serum Glutamic Oxaloacetic Transaminase, SGPT - Serum Glutamate Pyruvate Transaminase, ALP - Alkaline Phosphatase)

Out of 30 cases of liver disease patients 24(80%) cases had SGPT values more than 40 mg/dl. While in control group 4(13.33%) had SGPT values more than 40 mg/dl, showing sensitivity 80% and specificity of 86.67%. Out of 30 cases of liver disease patients 22(73.33%) cases had SGOT values more than 40 mg/dl. While in control group 10(33.33%) had SGOT values more than 40 mg/dl, showing sensitivity 73.33% and specificity of 66.67%. Our study is in accordance with the study of Ogunke et al14,15,16 who showed that there was close relationship between the severity of cirrhosis and level of serum cholinesterase enzyme.

5. Discussion

Estimation of the level of activity of the cholinesterase found in serum was first suggested by McArdle (1940)13, as a useful means for differentiating hepatic from post-hepatic jaundice. The evidence which has accumulated suggests that cholinesterase activity is an assessment indicator for liver function in patients with liver disease.

Present study was conducted to find out the effectiveness of serum cholinesterase enzyme to correctly diagnose liver diseases. Serum cholinesterase appears to originate in the liver and is closely associated with the synthesis of serum albumin17,18,19. It has been shown that even very low pre-liver transplant serum cholinesterase levels improve by second week after a successful liver transplantation, thus confirming the hepatic origin of this enzyme. It is synthesized mainly in hepatocytes and released into the blood. Serum ChE activity is reduced in liver dysfunction due to reduced synthesis; in contrast to other serum enzymes associated with the clinical assessment of liver function whose content increases as a result of increased release from their cellular sources following cell membrane damage.

Data from study conducted by Khan17 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.

Our study is in accordance with the study of Ogunkeye14,20, he also reported lower level of serum cholinesterase level in liver disease patients. William Burnett also found serum cholinesterase is useful both as a liver function test and in the diagnosis of jaundice16.

Ramachandran et al found Median serum ChE in cirrhosis was 1590 IU/L (110-8143) compared to controls 7886 IU/L (2022-21673), p<0.001. Serum ChE levels below 3506 had a 98.7% sensitivity and 80.3% specificity in predicting cirrhosis found serum ChE is an excellent biomarker of cirrhosis with good sensitivity and specificity20.

6. Conclusion

In this study comparison of serum cholinesterase levels versus conventional liver function tests was done 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. Serum cholinesterase had 90% sensitivity and 100% specificity and it must be added as a routine diagnostic test beside other liver function tests for investigation of liver dysfunctions. Larger sample size study should be carried out to reconfirm the conclusion.

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