Serum cholinesterase activity helps to distinguish between liver cirrhosis, hepatocellular carcinoma and chronic hepatitis C virus in Egyptian patient

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Abstract: Cholinesterase (ChE) 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 activities increases as the result of increased release from their cellular sources following cell membrane damage. So we can use ChE as a distinguishing enzyme in liver diseases. This study aimed to compare serum cholinesterase activities (ChE) of patients with chronic hepatitis C virus (CHC), liver cirrhosis (LC), hepatocellular carcinoma (HCC) and control to determine if serum cholinesterase activity can help in distinguishing these groups of subjects. **Materials and Methods:** serum sample from 60 subjects (38 male; 22 female; the age ranged between 20 and 70 years) divided into four groups 15 patients in each, chronic hepatitis C virus (CHC), liver cirrhosis (LC), hepatocellular carcinoma (HCC) and control. Liver tests including cholinesterase AST, ALT, Albumin and AFP were done. **Result:** Cholinesterase activity is decreased in LC and HCC as compared to CHC and control. HCC and LC are very high significantly different as compared to control, but insignificantly different as compared to each other. These results suggest that the determination of serum cholinesterase activity is a cost-effective diagnostic means of differentiating between liver diseases. **Conclusion:** We can use ChE to distinguish between CHC and HCC and between CHC and LC but we cannot use ChE to distinguish between HCC and LC, so ChE consider a cheap and easy test for distinguishing.

[EL Zwahry E, Ahmed DFI, El Masry M and Gad NA. **Serum cholinesterase activity helps to distinguish between liver cirrhosis, hepatocellular carcinoma and chronic hepatitis C virus in Egyptian patient.** *Researcher* 2013;5(7):33-37]. (ISSN: 1553-9865). http://www.sciencepub.net/researcher. 6

Key words: HCV; Cholinesterase; Liver; Diseases; Egypt

1. Introduction

Cholinesterase (ChE) is a family of enzymes that catalyze the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation (Wang and Tang 2005). Human cholinesterase is a glycoprotein. The 2 types of cholinesterases found in the human blood are acetyl cholinesterase ("true" cholinesterase) in red cells and butyrylcholinesterase (non-specific, pseudo cholinesterase) in serum (Brimijoin and Koenigsberger 1999).

Liver disease is any disturbance of liver function that causes illness. The liver is responsible for many critical functions within the body and should it become diseased or injured, the loss of those functions can cause significant damage to the body. Liver disease is also referred to as hepatic disease (Sheila and James 2002). Cholinesterase ChE 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 a result of increased release from their cellular sources following cell membrane damage (Moss and Henderson 1999).

Butyrylcholinesterase is synthesized in the liver. The serum butyrylcholinesterase level has been cross-sectionally reported to be higher in patients with diabetes, hyperlipidemia, obesity, and fatty liver than in those without them. It is not known whether serum butyrylcholinesterase is associated with the risk of future type 2 diabetes (Sato et al. 2013).

Biochemical tests for the assessment of liver function (commonly referred to as liver function tests), comprising serum aspartate (AST) and alanine (ALT) transaminases, albumin (Alb) and Alpha-fetoprotein (AFP) are often abnormal in patients with clinical problems other than liver dysfunction (Weisinger 2000).

As a result, none of these tests can individually confirm liver dysfunction. The predominant hepatic source of serum ChE, 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 other traditional liver function tests (Brown et al, 1981).

2. Patients and Methods

Serum samples were analyzed from 60 subjects (38 male; 22 female; the age ranged between 20 and 70 years) who presented to the Nile Badrawi hospital and

divided into four groups:

Group 1: control subjects (15 cases).

Group 2: chronic hepatitis C subjects (CHC) are positive to hepatitis C virus by RNA PCR (riboneocleic acid polymerase chain reaction) (15 cases).

Group 3: hepato-cellular carcinoma subjects (HCC) are positive to hepatitis C virus by RNA PCR, increased in AFP and decreased in ALB levels (15 cases).

Group 4: liver cirrhosis subjects (LC) are positive to hepatitis C viruse by RNA PCR and decreased in ALB level (15 cases).

Determination of serum Aspartate transferase (AST) and serum Alanine transferase (ALT)

The activity of AST and ALT were determined according to the method described by Thomas (1998) by using kit of OLYMPUS reagent (OLYMPUS GmbH, Germany).

Determination of serum Albumin (ALB)

The activity of ALB was determined according to the method described by Grant and Silverman (1987) by using kit of OLYMPUS reagent (OLYMPUS GmbH, Germany).

Determination of serum Alpha-fetoprotien (AFP)

It was determined according to method described by Ruoslahti and Engvall (1979) by using kit of ARCHITECT immunoassay.

Determination of serum cholinesterase (ChE)

It was determined according to Ellman's kinetic method (1959) by using kit of diamond diagnostic. A photometer Model BM 750 from Roach Company was used.

Statistical analysis

Data were analyzed statistically using student t-test under Excel software of Microsoft office program.

3. Results

A significant increase in ALT and AST ($P<0.001$) in the three groups when compared with control, on the other hand ChE activity shows a significant decrease ($P<0.001$) when compared to control in the three groups.

Also there is significant decrease in albumin in all groups compared with control with different probabilities. But AFP shows a significant increase ($P<0.001$) in HCC group when compared with control.

In another direction, AST shows significant increase ($P<0.001$) in HCC and LC groups when compared with the CHC group. Where, albumin and ChE shows significant decrease ($P<0.001$) in HCC and LC groups comparing to CHC group.

Also, AFP shows significant increase ($P<0.001$) in HCC group comparing with CHC group. ALT shows significant decrease ($P<0.01$) in LC group comparing to CHC group. By comparing HCC group to LC group, ALB and AFP decreased significantly in LC group with different propabilities.

From the results you can notice that the highest level for cholinesterase was in chronic hepatitis C virus (CHC) group and the lowest level for cholinesterase was in hepatocellular carcinoma (HCC) group.

4. Discussion

Albumin concentration may be reduced for reasons other than failure of liver synthesis. However, when many of the liver function tests are abnormal at the same time in a patient, liver disease is a more probable clinical diagnosis (Whicher and Spence, 1987).

Serum cholinesterase activity which is reduced in liver dysfunction due to reduced synthesis recovers with improvement of liver function (Brown and Kalow, 1981).

Rathanam and Ramanna (2007) reported that the (AST) (GGT) enzymes were widely used as markers for liver disorders, the ubiquitous enzyme butyrylcholinesterase (BChE), synthesized in liver was also used as marker in the assessment of liver pathophysiology. This BChE enzyme in addition to its esterase activity has yet another enzymatic function designated as aryl acylamidase (AAA) activity. It is determined *in vitro* based on the hydrolysis of the synthetic substrate o-nitroacetanilide.

Toshikazu and Masahide (1999) compared *Aleuria aurantia* lectin (AAL)-reactive serum cholinesterase (ChE) activity increases in liver cirrhosis (LC) and hepato-cellular carcinoma (HCC) with chronic hepatitis (CH) and normal controls (NC), and measurement of AAL-reactive ChE activity is useful in discriminating LC from CHC.

The prevalence of inheritable atypical cholinesterase genes in many populations, as reported in various studies (Steegm¨uller, 1975 and Pinto, 1996) is so low as not to invalidate the usefulness of the findings in this study for general clinical application. A single determination of serum cholinesterase activity in an individual can make a full liver function test profile unnecessary, when there is the need to distinguish between liver disease and other clinical problems associated with aberration of liver function tests. We therefore suggest that serum cholinesterase activity determination in this way is more cost effective than performing the standard liver function tests.

Table 1. Mean±SE of AST, ALT, ALB, AFP and ChE levels among all studied subjects.

groups		AST U/L	ALT U/L	ALB g/dl	AFP Iu/ml	ChE U/ml
Control	**Mean ±S.E.**	19 +-7.10	19 +-5.1	4.5 +- 0.61	2.5 +-0.62	4548 +-67
CHC	**Mean ±S.E.**	67*** +-16.3	82*** +-43.3	3.9* +-0.42	2.9 +-0.56	3782*** +-157
LC	**Mean ±S.E.**	99***### +-27.4	54***## +-6.9	2.3***### $$ +-0.38	4.4$$$ +-1.32	2641***### +-210
HCC	**Mean ±S.E.**	102***### +-25.0	67*** +-20.3	2.6**### +-0.44	35***### +-21.3	2553***### +-102

The statistical analysis is performed using t-test
*=significant comparing to control; # = significant comparing to CHC; $ = significant comparing to HCC
* Significant = (P<0.05)
Highly significant=(P<0.01); * Very highly significant = (P<0.001)
# and $ as the same * in degree of significant
CHC= chronic hepatitis C virus; HCC= hepatocellular carsinoma; LC= liver cirrhosis

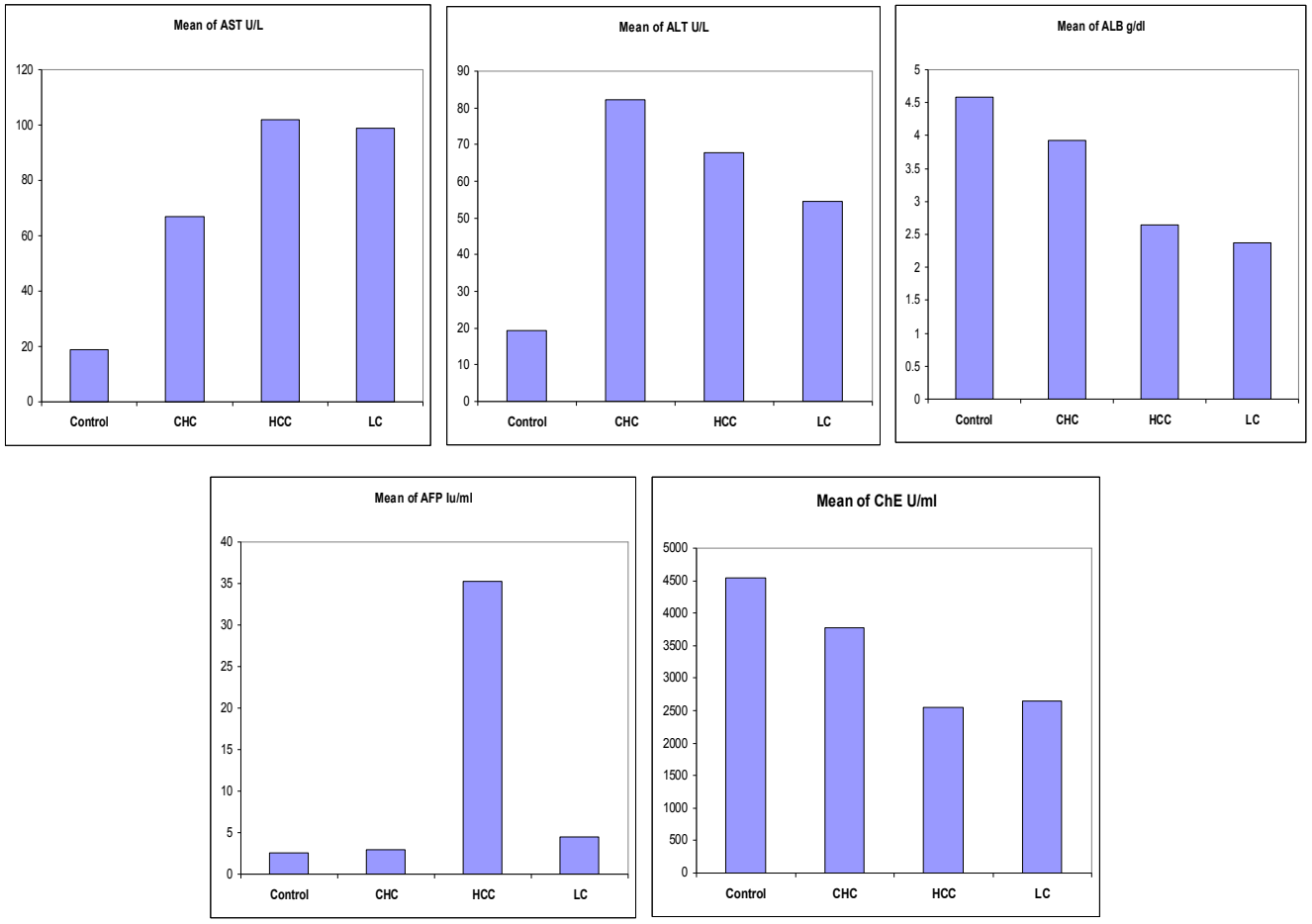

Figure 1. AST, ALT, ALB, and ChE among all studied subjects.

The traditional liver function tests which we selected for study are sometimes abnormal in illnesses that are unconnected with liver dysfunction. The activities of serum transaminases may be raised due to increased release from non-liver tissue sources in various pathologies (Rej, 1989). Albumin concentration may be reduced for reasons other than failure of liver synthesis (Whicher and Spence, 1987). However, when many of the liver function tests are abnormal at the same time in a patient, liver disease is a more probable clinical diagnosis. ChE activity which is reduced in liver dysfunction due to reduced synthesis recovers with improvement of liver function (Brown et al., 1981). This recovery is quicker than that observed following recovery from the effects of organo-phosphorus poisoning; another clinical condition in which marked reduction in ChE activity is a diagnostic feature (Namba, 1971).

In Our study CHC is very high significantly different as compared to HCC, LC and control subjects (Table 2). HCC are very high significantly different as compared to CHC and control but insignificantly different as compared to LC subjects (Table 1). LC are very high significantly different as compared to CHC and control but insignificantly different as compared HCC subjects (Table 1).

Chronic liver disease (CLD), such as hepatitis C, is a progressive disease consisting of the destruction and regeneration of the liver parenchyma, leading to fibrosis and cirrhosis Maruyama et al. (2013). In accordance to Ogunkeye et al., 2009), the diagnostic usefulness of a single determination of serum cholinesterase activity to distinguish between overt liver disease and non-liver disease clinical problems in which a few of the traditional liver function tests were abnormal was assessed. Using three groups of subjects comprising liver disease, non-liver disease, and healthy controls, they have shown that serum cholinesterase activity helped to distinguish between liver disease and non-liver disease in subjects who had abnormality of a few liver function tests. Serum cholinesterase activity helped also to distinguish between the liver disease subjects and healthy controls. There was no statistically significant difference between the mean serum cholinesterase activities of non-liver disease subjects and healthy controls. They suggest that determination of serum cholinesterase activity is a cost-effective diagnostic means of differentiating between overt liver disease and non-liver diseases where there may be aberration of some liver function tests.

Ogunkeye and Roluga (2006) compared serum cholinesterase activities of a group of type 2 diabetic patients showing clinical evidence of non-alcoholic fatty liver disease to those of age and sex-matched type 2 diabetics who showed no evidence of liver disease, and healthy control subjects to determine, if serum cholinesterase can be used to diagnose nonalcoholic fatty liver disease in type 2 diabetic patients. The investigators found that the mean serum cholinesterase activity in diabetics with nonalcoholic fatty liver disease was found to be statistically significantly lower than in diabetics without liver disease and in healthy subjects. There was also no statistically significant difference between the mean values of serum cholinesterase activities of non-liver disease diabetics and healthy control subjects.

Kotoh et al., (2012) reported that serum albumin levels were higher in patients with alcoholic cirrhosis and alcohol consumption should be carefully considered when evaluating hepatic functional reserve.

Chronic liver disease (CLD), such as hepatitis C, is a progressive disease consisting of the destruction and regeneration of the liver parenchyma, leading to fibrosis and cirrhosis.(Maruyama et al. 2013).

The study has shown that ChE activity singly, can help to distinguish between LC, HCC, CHC and control, suggests that ChE activity measurement can serve as a low cost means of distinguishing between hepatic cases. A single determination of ChE activity in an individual can make a full liver function test profile unnecessary, when there is the need to distinguish between liver disease and other clinical problems associated with aberration of liver function tests. We therefore suggest that ChE activity determination in this way is more cost effective than performing the standard liver function tests.

5. Conclusion

Cholinesterase (ChE) can use to distinguish between CHC and HCC and between CHC and LC but we cannot use ChE to distinguish between HCC and LC, so ChE consider a cheap and easy test for distinguishing.

6. Recommendation

We recommend with using ChE test to distinguish between different types of liver diseases.

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4/10/2013