CHANGES OF SERUM BILE ACIDS IN LIVER DISEASES

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ABSTRACT

The present study was carried out to evaluate the clinical usefulness of measuring total serum bile acids concentrations as a diagnostic test for hepatobiliary diseases. It is measured by an enzymatic method under fasting conditions in 120 apparently healthy control subjects and 140(79 (56.43%) males and 61(43.57%) females) patients with various forms of hepatobiliary diseases. The patient's diagnoses were based on clinical, biochemical, radiological, serological and histological grounds. The study showed a highly significant increase in total serum bile acids levels in all types of liver disease patients groups (icteric and anicteric) (P<0.001), even when other liver tests are normal. There were no significant differences (P> 0.05) in the serum levels of total bile acids in patients groups according to their gender and residence. The highest incidences of hepatobiliary diseases were in males and urban patients groups as compared with females and rural patients groups respectively. The study has illustrated moderate correlations between the concentrations of total bile acids with those for any the other liver function tests. Highly significant, positive and moderate correlations were observed with total bilirubin concentration (r = 0.659, P < 0.01). On the other hand serum total bile acids were negatively correlated with serum albumin (r = - 0.104).

INTRODUCTION

Bile acids (BAs) are a group of water-soluble steroids formed during the catabolism of cholesterol, and synthesized in the hepatocytes of the liver. They have 24 carbon atoms and are abbreviated as C_{24} bile acids. Bile acids or bile salts have a unique and fascinating molecular structure derived from a saturated tetracyclic hydrocarbon perhydrocyclopentano-phenanthrene system, usually known as the steroid nucleus.[1] Five major physiological functions of bile acids are now well established. First and most important is the elimination of cholesterol. Second is lipid transport in the form of mixed micelles. Unless bile acids are present in micellar form, fat-soluble vitamins (A, D, E, and K) will not be absorbed. [2] The third and fourth functions of bile acids are stimulation of bile flow and stimulation of biliary phospholipids secretion. Fifth is negative feedback regulation of bile acids and cholesterol biosynthesis.[2,3] Fasting serum total bile acids determinations were used clinically in the diagnosis and prognosis of liver disease in conjunction with liver function tests. Because of the increased sensitivity of serum total bile acids determination as compared to liver function tests, serum total bile acid testing offers significant additional diagnostic information concerning liver function, especially in minor hepatic derangements. Plasma total bile acids levels are a sensitive indicator of liver function in all species, reflecting hepatic synthesis, secretion, and re-absorptive functions. [4,5] Liver injury as a result of occupational or environmental exposure to a wide variety of chemical substances can be determined to a much finer degree by serum total bile acid than by standard liver enzymes, especially when the liver has been only slightly damaged. Liver function tests sometimes lack sensitivity and specificity, and alone are inefficient in detecting the presence and defining the nature of hepatobiliary disease. [4,6]

The aims of the study were, firstly, to study the changes in total serum bile acid concentrations and liver function tests in patients with liver disease.

Secondly, to study the possible correlation and interaction between total serum bile acid concentrations and other parameters included in liver function tests, and also, to evaluate whether total serum bile acids measurements could serve as a valuable indicator in detecting liver disease.

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METHODS AND PATIENTS

Patients:

A case control study conducted from 1/11/2008 to 31/10/2009 during which 140 samples of liver diseases patients were collected (79 males and 61 females) from Al-Sader teaching hospital, Public Health Department and Al-Faiha general hospital. Who are their age range between 5 to 70 years and a mean of their age were (36.4±19) years. They were categorized into two main subdivisions, clear distinction between males and females, as hepatobiliary diseases patients and controls. The patient's diagnoses were based on clinical, biochemical, radiological, serological and histological grounds. The control group consisted of 120 (63 males and 57 females) healthy, asymptomatic, clinically, radiological and biochemically normal subjects and none had a previous history of liver disease. Their age range between 5 to 70 years and a mean of their age were (37.40 ±17.12) years.

Methods:

After an average fast of 10-14 hours (5) ml of venous blood samples were taken from both patients and control subjects by a sterile disposable syringe then collected in plain plastic tubes. The serum was separated immediately after withdrawal and immediately analyzed or stored in plain tubes in a deep freeze $(-20)^0$C, until used. They were investigated for different parameters of serum levels for liver function tests which include: Total bile acids, Total bilirubin, Direct and indirect bilirubin, Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphate, Gamma-glutamyltransferase, Protein, Albumin and Globulin. All diagnostic kits were purchased from Randox, Roche, BioMerieux and Biolabo.

RESULTS

Table-1, shows no significant differences in serum of T. Bile acids, T.S. Bilirubin, S.AST, S. ALT, S.APL, and S.GGT in all liver diseases among patients in relation to residence and gender (P>0.05), while there was a highly significant decrease in serum level of albumin in females compared to males (P < 0.01).

Table 1.Comparison of serum levels biochemical parameters among patients in relation to residence and gender.

The results expressed as mean ± SD.

Parameters	Gender		Residence	
	Male	Female	Urban	Rural
No.	79	61	73	67
S.T.Bile acids (µmol /L)	38.45±4.32	42.45±4.80	21.56±2.40	26.20± 3.68
S.T.billirubin (µmol /L)	83.96±10.64	90.21±11.38	51.58±6.17	53.53±7.53
S.AST (U/L)	53.36±5.26	59.18±6.82	39.02±3.11	35.76±4.45
S.ALT (U/L)	51.01±5.41	49.51±7.12	31.29±2.79	36.07±5.00
S.ALP (U/L)	165.34±17.32	153.86±12.79	114.89±9.21	108.47±10.2
S.GGT (U/L)	129.49±11.79	126.35±10.69	94.02±7.02	84.50±7.04
S.T. Protein (g/l)	68.60±0.44	67.22±0.52*	69.79±0.31	69.16±o.41
S. Albumin (g/l)	38.32±0.65	35.51±0.73**	39.63±0.45	39.31±0.50
S. Globulin (g/l)	31.05±0.83	31.80±0.60	30.68±0.45	29.75±0.34

***, **Significantly differences as compared with male subjects (P < 0.05, P < 0.01, respectively).**

(Table-2), shows that, serum total bile acid concentrations were above normal (highly significant increase, P< 0.001) in all types of liver disease among patients groups. Viral hepatitis A and Alcoholic hepatitis groups showed a highly significant increase in T. S. Bile acids, T. S. Bilirubin, serum Liver enzymes (AST, ALT. ALP and GGT) (P < 0.001). On the other hand, there was a significant decrease in serum level of T. proteins and albumin (P< 0.05). Viral hepatitis B group showed a highly significant increase in T. S. Bile acids, T. S. Bilirubin, AST and ALT (P < 0.001). On the other hand, there was no significant differences in serum level of ALP, GGT, T. proteins, albumin and S. globulins (P > 0.05).

Viral hepatitis C group showed a highly significant increase in T. S. Bile acids and T. S. Bilirubin (P < 0.001). On the other hand, there was no significant differences in serum level of AST, ALT, ALP, GGT and S. globulins (P > 0.05). There was a highly significant decrease in serum level of T. proteins and albumin (P < 0.01).

Obstructive Jaundice, Secondary Liver Cancer and Liver Cirrhosis groups showed a highly significant increases in T. S. Bile acids, T. S. Bilirubin, serum Liver enzymes and S. globulins (P < 0.001). On the other hand, there was a highly significant decrease in serum level of T. protein and albumin (P<0.001).

Table 2. Serum T. Bile acids and other parameters in patients with liver disease.

Para.	Control	Hepatitis A	Hepatitis B	Hepatitis C	Obstruc Jaundice	Secondary L. Cancer	Liver Cirrhosis	Alcoholic Hepatitis	Uncon.Hyperbilirubinaemia
No.	120	27	15	21	19	17	18	4	19
S.T. BA (µmol/L)	3.8057 ±0.14	80.18 ±6.14***	8.18 ±2.41***	8.22 ±2.61***	84.08 ±5.26***	28.21 ±4.09***	48.2 ±5.64***	36.97 ±9.75***-	4.19 ±0.3
T. Bil. (µmol/L)	12.31 ±0.23	131.89 ±11.21***	18.81 ±3.55***	15.19 ±0.68***	207.53 ±24.22***	38.11 ±11.27***	95.33 ±20.37***	168.99 ±98.24***	52.15 ±3.69***
S.AST (U/L)	16.51 ±0.57	88.04 ±11.38***	26.60 ±8.69**	19.93 ±3.17	92.87 ±10.66***	50.35 ±11.0***	78.27 ±9.39***	59.32 ±14.25***	19.14 ±1.87
S.ALT (U/L)	13.19 ±o.44	117.04 ±11.63***	25.59 ±10.86**	14.24 ±1.51	77.54 ±8.82***	27.33 ±5.6 1***	46.53 ±3.65***	39.55 ±6.7***	14.38 ±1.51
S.ALP (U/L)	56.05 ±1.23	123.57 ±8.39***	63.15 ±7.71	58.59 ±7.91	308.42 ±27.16***	292.86 ±24.47***	202.26 ±22.48***	326.07 ±170.19***	60.51 ±4.09
S.GGT (U/L)	45.98 ±1.12	104.44 ±5.41***	43.32 ±6.53	51.46 ±10.39	228.96 ±17.05***	211.26 ±19.98***	184.13 ±21.28***	281.24 ±54.11***	52.92 ±3.49
S.T. Prot. (g/l)	71.33 ±0.29	69.85 ±0.55*	70.96 ±1.02	66.8 ±0.82***	67.59 ±1.01***	66.97 ±1.13***	64.41 ±0.87***	67.54 ±1.86*	69.18 ±0.53**
S. Alb. (g/l)	42.31 ±0.26	41.08 ±0.36*	42.58 ±1.0	37.63 ±0.75***	36.77 ±0. 68***	31.05 ±0.61***	28.0 ±1.11***	38.07 ±4.45*	40.67 ±0.71*
S. Glob. (g/l)	29.06 ±0.16	30.81 ±2.06	28.38 ±0.95	29.62 ±0.81	30.81 ±0.70**	35.91 ±0.92***	36.48 ±1.18***	29.45 ±2.77	28.58 ±0.4

The results expressed as mean ± SD
(*, **, ***significantly differences as compared with healthy subjects (P < 0.05, P < 0.01, P < 0.001, respectively).

Table-3, presents the correlation analysis between serum T. Bile acids and the other biochemical parameters within the present groups and in all patients groups of liver diseases. In all liver patients, the correlation were highest, positive highly significant with serum total bilirubin, (r = 0.659, P<0.01). Next to S.T. bilirubin, alanine aminotransferase showed the highest, positive and highly significant correlation with serum T. Bile acids, (r = 0.593, P < 0.01). On other hand, total serum bile acids was positive highly significant correlated with S.AST, S.ALP and S.GGT (P<0.01, r = 0.516, r = 0.462 and r = 0.501) respectively. Also total serum bile acids showed negative correlation with serum total protein (r = - 0.011) and albumin (r = - 0.104).

Table 3. Correlation coefficient (r) between Serum T. Bile acids and other liver function tests in liver diseases.

Patients Groups	No.	S.T. BIL.	S. AST	S. ALT	S. ALP	S.GGT	S.T. PROT	S. ALB.
Hepatitis A	27	0.251	-0.267	-0.212	0.15	0.452*	-0.366	-0.212
Hepatitis B	15	0.96**	0.97**	0.981**	0.836**	0.86**	0.213	-0.118
Hepatitis C	21	0.839**	0.972**	0.913**	0.894**	0.946**	0.185	-0.755**
Obstructive Jaundice	19	0.546*	-0.086	-0.018	-0.113	0.002	-0.085	-0.470*
Secondary Liver Cancer	17	0.659**	0.518*	0.517*	0.765**	0.658**	0.362	0.07
Liver cirrhosis	18	0.252	0.19	0.212	0.329	0.381	0.191	-0.197
Alcoholic Hepatitis	4	0.015	-0.231	0.106	0.087	0.484	0.844	0.634
Uncon. Hyperbilirubinaemia	19	-0.157	-0.004	-0.254	0.084	-0.116	0.161	0.342
All liver disease patients	140	0.659**	0.516**	0.593**	0.462**	0.501**	-0.011	-0.104

DISCUSSION

Measurement of total serum bile acids is a relatively easy, safe, and rapid means of assessing hepatic function. Bile acids are stable in serum for long periods of time and can be frozen. [7] This study has shown that fasting total serum bile acids concentrations in blood were significantly increased (P<0.001) in all types of hepatobiliary disease patients who were icteric and anicteric, (hepatitis A, 80.18±6.14; hepatitis B, 8.18±2.41; hepatitis C, 8.22±2.61; obstructive jaundice, 84.08±5.26; secondary liver cancer, 28.21 ± 4.09; liver cirrhosis, 48.2 ± 5.64; alcoholic hepatitis, 36.97 ± 9.75), even when fasting, the serum concentrations levels of other liver function tests are normal. This is in contrast to normal control subjects whose bile acid concentrations did not raise outside the normal fasting range (3.8057 ± 0.14), as shown in (Table-2). An increase in serum bile acid values will be expected in the presence of portosystemic shunting; any compromises of liver function or damaged hepatocytes which are unable to extract the bile acids from the portal blood. It is also apparent that bile acids leak directly from the liver to the systemic circulation, very high serum bile acid concentrations are found in patients with extrahepatic obstruction. There was a highly significant decrease (P<0.001) in levels of serum albumin for all patients groups, (Table-2), which may reflect reduced capacity of liver for synthesis functions. In addition to that, there was a highly significant decrease (P < 0.001) in levels of serum albumin for all patients groups, as compared with controls, as seen in (Table-2), which may reflect reduced capacity of liver for synthesis functions. Our study confirms and extends the conclusion of previous reports that the concentration of total bile acids in patients is highly significant and not highly correlated with other liver tests. It was significantly correlated with total serum bilirubin. We were found the estimation of total bile acids to be more sensitive than is total bilirubin.[8,9] Using discriminant analysis, we found that the bile acids tests added information to a panel of tests examined previously. Different parenchymal disorder and cholestatic syndrome will affect composition, concentration and kinetics of the bile acids in blood. Accordingly, we found that the total serum bile acids were some time moderately correlated with other liver function tests thought to express quite different liver functions' as shown in (Table-3). Our results, showed a highly significant increase of total serum bile acids and serum total bilirubin (P<0.001) in all viral hepatitis patients (icteric and anicteric viral hepatitis A,B and C), as compared with control, as shown in **(**Table-2). These results were in agreements with other studies by researchers in different parts of worlds.[10] This increases serum parameters levels due to the damage of liver cells. In addition to their value as a screening test for hepatobiliary disease, serum bile acid determinations are useful in following the progress of viral hepatitis. In addition, in all viral hepatitis types, serum total bile acids were negatively correlated with albumin, which may reflect reduced capacity of liver for synthesis functions of albumin due to liver cells damage. In addition to their value as a screening test for hepatobiliary disease, serum bile acid determinations are useful in following the progress of viral hepatitis. Serum bile acid determinations are useful in judging the response to therapy of patients with chronic active liver disease, and have demonstrated that they are superior to liver biopsy (which is invasive and dangerous procedure in liver diseases) in predicting those patients who subsequently relapsed following biochemical and histological resolution.[4,11] Toshihide Sima el al,[4] recently reported that serum total bile acids levels is a sensitive indicator of hepatic histological improvement in chronic hepatitis C patients responding to interferon treatment. A decrease in serum total bile acids levels reflects histological improvement in the liver more precisely than change of other liver function

tests values following interferon therapy. In the current study, cholestasis, secondary liver cancer, liver cirrhosis and alcoholic liver groups showed highly significant increases ($P<0.001$) in total serum bile acids, which was to be expected as they often had histological liver disease, (Table-2). In liver cirrhosis, the total serum bile acids may even be more sensitive than aminotransferases and other serum enzymes. With respect to liver metastases, our results were showing substantial elevation of total serum bile acids and serum alkaline phosphatase. In addition, a highly significant correlation between total serum bile acids and serum alkaline phosphatase and gama-glutamyl transferase is demonstrable.[11-13] Patients with unconjugated hyperbilirubinaemia the serum bile acid concentrations were normal (4.19 ± 0.3; $P > 0.05$) as liver enzymes (AST, ALT, GGT, ALP), as compared with control, which was to be expected as they had no histological liver disease, (Table-2). Our results also showed negaitive correlations were found between total serum bile acids and S.T.bilirubin ($r = - 0.157$). These results were in agreements with other studies. Serum bile acid concentrations in patients with Gilbert's disease are normal and this measurement (if available) is the best test for differentiating Gilbert's disease from hyperbilirubinaemia due to hepatic disease. [14]

CONCLUSIONS

1. Serum total bile acids test is a very sensitive and specific indicator of hepatic function, the most valuable applications of the test are to identify impaired liver function, to confirm or deny the presence of significant hepatic disease.
2. The test is non-invasive, easy to perform by the present enzymatic method, convenient to submit to a regional laboratory and when used for appropriate reasons, relatively simple to interpret.
3. Serum bile acid determinations increase the diagnostic and discriminant capacities of liver function tests; we also found the total serum bile acids added new information the panel of tests used previously.
4. Total bile acids in fasting patients are positive, moderate and highly significant correlated with other liver tests and negatively correlated with serum albumin.

REFERENCES

1. Monolio TA, Burke GL, Savage PJ, et al. Sex and race related differences in liver associated serum chemistry tests in young adult in the cardia study. Clin Chem 1992; 38: 1853- 1859.
2. Vlahcevic ZR, Heuman DM, Hylemon PB. Regulation of bile acid synthesis. Hepatology 1991; 13: 590- 600.
3. Carey MC, Duane WC. Enterohepatic circulation. In: Arias IM, Boyer JL, Fausto N, Jakoby WB, Schachter DA, Shafritz DA, eds. The Liver: Biology and Pathobiology. 3rd ed. New York, NY: Raven Press; 1994: 719- 768.
4. Toshihide Shima, et al. J. Gastroenterology and hepatology 2000; 15: 294-299.
5. Hofmann AF. The continuing importance of bile acids in liver and intestinal disease. Arch Intern Med 1999; 159:2647–2658.
6. Sherlock S. Overview of chronic cholestatic conditions in adults: terminology and definitions. Clin Liver Dis 1998; 2: 217- 234.
7. Kytzia HJ. Reference intervals for GGT according of the new IFCC 37 ^{0}C reference procedure. Abstracts: Congress of Clinical Chemistry and Laboratory Medicine A 103, Jena, Oct. 2005.
8. Kabicek P. Importance of serum bile acids determination in adolescents with juvenile hyperbilirubinaemia. Cent Eur J Public Health 2004; 12: 102- 109.
9. Polkowski W, Kudlicka A, Wallner G, Chrzastek- Spruch H. Range of serum bile acid concentrations in neonates, infants, older children, and in adults. Med Sci Monit 2001; 7 Suppl 1: 268- 270.
10. Chen W, Liu J, Gluud C. Bile acids for viral hepatitis. Cochrane data base syst. Rev. 2007; 4: CD 3181.
11. Azer SA, Coverdale, SA, Byth K, Farrell GC, and Stacey NH J. Sequential changes in serum levels of individual bile acids in patients with chronic cholestatic liver disease. Gastroenterol Hepatol 1996; 11: 208- 215.
12. Dong Y , Li J, Xiang S, S,. Bao Z, Fan H, Yang F, Li Z 12. Serum bile acid chromatography to the diagnoses of liver diseases. 1997; 28: 69- 72.
13. Trinchet JC., Gerhardt Tomos MF, Balkau B, Munz C, Poupon RE. Serum bile acids and cholestasis in alcoholic hepatitis. Relationship with usual liver tests and histological features.J Hepatol 1994; 21: 235- 240.
14. Center SA, ManWarren T, Slater MR, Wilentz E. Evaluation of twelve-hour preprandial and two-hour postprandial serum bile acids concentration for diagnosis of hepatobiliary disease. J Am Vet Med Assoc 1991; 199: 217- 226.