Article

The Assessment of Serum Cytokeratin 18 and Liver Function Tests in Chronic hepatitis B patients.

Zinah Abbass Ali1, Hawraa Mudheher Kadhim 2 , Samar Hasan Shammar2 and Zainab mohsen najm2

1Department of Clinical Biochemistry, College of Medicine, University of Babylon

2University of Babylon

Abstract

Background

Chronic hepatitis is a clinical and pathological disorder, some biochemical testes like serum Alanine Amino-transferase (ALT), Aspartate Amino-transferase (AST) and total serum bilirubin (TSB) used to monitoring hepatitis diseases. Albumin levels in serum fall with liver disease progression, representing decreased synthesis. Prothrombin time evaluate the level of converted prothrombin to thrombin and this represent the synthetic function of the liver. In acute on chronic liver

Disease non apoptotic pattern of cell death is predominant .It is confirmed by cleaved K18. Cytokeratin 18 was measured by ELISA

Objectives: This study aimed to measure the ALT ,AST ,TSB, Albumin ,PT and K18 levels inthe serum of Iraqi patients who were having Liver disease And study the correlation between the variables mentioned above

Materials and Methods: The study included approximately One hundred subjects, ages 20 to 60 year, who were split into two groups , Fifty patient with chronic hepatitisB virus,The second group comprises of 50 subjects who look to be in good health and serves as the control group.

Results

Serum levels of ALT, AST, TSB, PT and albumin displayed a significant increase in chronic hepatitis B virus group compare with control group .

Also, the current study revealed non-significant association of liver function tests with different age groups, except total serum Bilirubin values decline significantly with increasing age, and there was a significant gender difference in AST concentration. While, there was non-significant gender difference in further liver parameters.

Conclusion

Among chronic hepatitis B patientin Babylon province, abnormality of liver function tests in different age and gender groups; indicat the significant linke with the development of chronic hepatitis B virus.

Keywords: Chronic hepatitis B virus, Aspartate Aminotransferase, Alanine Aminotransferase, albumin, Total serum bilirubin and Prothrombin time.

Introduction

Any inflammation of the liver organ cells (like hepatocyte infection or dysfunction) known as Hepatitis. Some patients display no signs of hepatitis, while further patients have jaundice (yellow-colored skin), diarrhea, vomiting, headache, reduced appetite, and nausea. If the hepatitis recurs within a half-year period is called acute hepatitis or else, if the hepatitis continues for more than 6 months is called chronic hepatitis **[1]**.

Chronic hepatitis is a clinical and pathological disorder, it has numerous reasons and is illustrious by several degrees of hepatocellular necrosis. A focal areas for parenchymic necrosis and dropout, big lobular areas of confluent necrosis, anteroseptal or periportal part necrosis with or without a connection are the identification of necrosed and inflammed parts of the liver. At least 6 months without recovery, chronic hepatitis remains an unimproved disease **[2]**.

The risk factors for HBV viruses includes working in a healthcare setting, blood transfusion or organ transplantation without HBV screening, acupuncture, dialysis, tattooing, extended overseas travel, infusion bags, multiple-use of medication vials, intravenous drug use (IDU) and improperly sterilized surgical equipment **[3]**.

The risk for the development of acute HBV infection to chronic hepatitis B is the age of patients. Less than 5 % of adults suffer from chronic infection, while about 90 % of infants develop chronic infection **[4]**.

About 1.4 million deaths per year as a result of viral hepatitis. 90% of these mortality caused by HBV, whilst other hepatitis viruses responsible of the remaining 10% of mortality **[5]**.

The major cause of chronic liver-disease is chronic infection with the hepatitis B virus (HBV) and prevalent in several countries even with the worldwide application of vaccine **[6]**.

The liver enzyme, serum glutamic pyruvic transaminase (SGPT), it also known as (ALT) existing in the liver cytosol and in low concentration somewhere else. ALT leakages out into the bloodstream and ALT level raised in the blood, when hepatocytes are damaged **[7]**.

On the other hand, another liver enzyme is serum glutamic oxaloacetic transaminase (SGOT) also known as (AST), it can be found in the heart, liver, skeletal muscle, brain, kidney, lungs, pancreas, leukocytes and erythrocytes. Viral hepatitis, acute liver cell damage, another cases like myocardial infarction are the main causes of elevated AST level **[8]**.

For the liver, ALT is present in the cytoplasm only, while AST is present in the cytoplasm and mitochondria, therefore, for hepatic damage ALT elevation is more specific **[9]**.

Bilirubin is the end product of heam breakdown during normal red blood cells damage, then bilirubin excreted into the bile. Most of bilirubin will remove from the body (if the liver is healthy), but in hepatocyte damage it can cause jaundice

(bilirubin leak out of liver into the bloodstream lead to elevated serum bilirubin level) **[10]**.

The synthesis of albumin is the main funtion of the liver. Around 10 g is synthesise and secrete every day. Albumin levels in serum fall with liver disease progression, representing decreased synthesis. Several factors affected the level of albumin like the nutrition, catabolism, hormones, urinary and gastrointestinal losses. That mean, albumin concentration associate with chronic liver disease progression **[11]**.

Some biochemical testes like serum ALT, AST, and serum bilirubin used to monitoring hepatitis diseases. The markers demonstrated degree of hepatocyte injury include serum ALT and AST levels.

The most common causes of elevated serum bilirubin level are hepatic cell damage, intrahpatic and extrahepaticbiliary tract obstruction and hemolysis. Patients with hepatic failure may have a slow serum bilirubin levels elevation, with an increase of more or equally 1 time upper limit of normal in a day, and a difference phenomenon (i.e. bilirubin elevation and ALT and AST decrease) might be happen **[12]**. Cytokeratin 18 is an middle filament protein highly expresse in epithelial cells. When released into extracellular space, K18 can be used as biomarker. Apoptosis and necrosis are the two major approaches to hepatocyte cellular death. Caspase cleaved keratin 18 (CCK18) is considered to represent hepatocyte Apoptosis . It is a type I cytokeratin. It is, together with its filament parter keratin 8, perhps the most commonly found product of the intermediate filament gene family. They are expresse in single layer tissues of the body. Mtations in this gene have been linke to cryptogenic cirrhosis. Two trancript variant encodin the same proein have ben fond for this gene [13].

Materials and methods

Study design, As a case-control the study was designated. One hundred subjects enrolle in the present study. Fifty patients with chronic hepatitis B virus, for all patients complete history was taken, which include: body mass index ,age and sex.

Also, fifty apparently healthy subject as a control group .The patients and control age from 20 to 60 years.

Inclusion and Exclusion criteria

Patients with renal failure, autoimmune diseases (like Rheumatoid Arthritis), Obesity and Patients with cirrhosis and hepatocellular carcinoma, were excluded from the study

Statistical Analysis

The result of study were shown as mean ± SD, for the evaluation of data Student's t-test and the linear regression analysis were used**.** Confidence interval (CI) 95% and P value were used for expression of data. SPSS (version 20) were performed for statistical analyses. P value was considered to be significant at 0.05 or less.

Ethical approval

All participants in this study were informed before to collecting samples, and verbal agreement was obtained from each of them. The protocol of study and the information of subject and agreement form were revised and accepted by a local ethics committee in Babylon Medical College.

Chemicals and methods

1- AST and ALT concentration were calculate by Siemens Dimension device method.

2- Serum albumin concentration was calculated by Siemens Dimension device and BCG (Bromocresol Green) as the method.

3- Total serum bilirubin (TSB) concentration was calculated by Siemens Dimension device and sulfanilic acid as the method.

4- Prothrombin time (PT) is determined by SP-NORMOPLASTIN reagent.

5- Cytokeratin 18 by ELISA

Results

This study comprise of 100 adults entitle in two groups:

1- Patient with chronic hepatiti B virus (n=50)
2- Apparently healthy subject as a control group (n=50)

Age

The difference in age (as mean) represented non-significant changed in chronic hepatitis B patients compare with the control group (Table 1). According to age, the frequency distribution of patient with chronic hepatitis B was as following: 34 (68 %) cases from 20-40 years and 16 (32%) cases between 41-60 years.

sex

To avoid sex genome interference with the activity of liver enzymes interested in this study, the sex of chronic hepatitis B patients and control group was selected to be equal for both males and females, the percentage of males was 50 % and percentage of females was 50% for each patients and control, Table 1.

Body Mass Index (BMI)

Results in table-1 shows that patients with a normal body mass index were selected in this study.

The relationship of Liver function test with age and gender groups.

The results of the preent study showed significant differences among chronic hepatitis B patients and controls regarding the value of ALT, AST, TSB, PT ,K18 and Albumin (Table 1).

Also, the current study revealed non-significant association of liver function tests with different age groups, except total serum Bilirubin values decline significantly with increasing age, and there was a significant gender difference in

AST concentration. While, there was non-significant sex difference in other liver parameter, (Table 2).

Table (1): Liver Function Tests in the studied groups.

Parameters	Mean ± SD		P-value
	Chronic HBV group N=50	Control group N=50	
Age (Years)	38.72 ± 10.79	35.52 ± 11.92	>0.05
BMI	23.08 ± 1.69	22.66 ± 1.95	>0.05
AST (IU/L)	58.74±25.96	19.96±3.22	< 0.05
ALT (IU/L)	65.05±23.52	21.22±2.46	< 0.05
TSB (µmol/L)	24.19±7.17	12.64±2.39	< 0.05
Albumin (g/dl)	3.54±0.34	4.32±0.33	< 0.05
PT (second)	12.02±0.54	11.42±0.53	< 0.05
K18	265.04±10.81	303.09±20.7	<0.05

AST:Aspartate aminotransferase; ALT:Alanine aminotransferase; TSB:Total serum bilirubin; PT:Prothrombin time , Cytokeratin 18; SD:standard deviation; p < 0.05 is significant.

Table (2): Association of Liver Function Tests in Chronic Hepatitis B patients with age and sex.

Association of Liver Function Tests in CHB patients with age groups			
Parameters	Age 20-40 (years) N=34 (Mean ± SD)	Age 41-60 (years) N=16 (Mean ± SD)	P value
ALT (IU/L)	61.39 ± 24.84	72.81±18.83	> 0.05
AST (IU/L)	56.82 ± 26.67	62.83±24.69	> 0.05
TSB (µmol/L)	28.81 ± 4.4	22.01±7.2	< 0.05
Albumin (g/dl)	3.58 ± 0.35	3.46±0.32	> 0.05
PT (second)	12.02 ± 0.58	12.01 ± 0.46	> 0.05
CCK18 pg/ml	264.1±8.1	266.2±11.2	> 0.05

Association of Liver Function Tests in CHB patients with gender			
Parameters	**Male (N=25) (Mean ± SD)**	**Female (N=25) (Mean ± SD)**	**P value**
ALT (IU/L)	68.8 ± 24.04	61.3 ± 22.85	> 0.05
AST (IU/L)	67.08 ± 22.87	50.4 ± 26.6	< 0.05
TSB (µmol/L)	24.38 ± 7.13	24 ± 7.35	> 0.05
Albumin (g/dl)	3.59 ± 0.33	3.54 ± 0.36	> 0.05
PT (second)	11.95 ± 0.53	12.09 ± 0.54	> 0.05
CCK18 pg/ml	268 .4±7.1	263.2±7.9	> 0.05

.

Discussion

According to liver functional tests, there was a significant association between hepatitis and each of ALT, AST, TSB, PT and Albumin. Since hepatocytes and bile duct cells (cholangiocytes) are the two different types of cells in liver tissue, ALT and AST are enzymes inside the hepatocytes and released into the circulation when there is hepatocellular inflammation or injury. On the other hand, the production of serum bilirubin is elevated during inflammation of cholangiocytes, which may be released during intrahepatic (or extrahepatic) bile duct obstruction or inflammation **[14]**.

Our finding is concomitant greatly with Al-Haidary B *et al*., 2008 **[15]**, who showed that all liver function tests raise in all hepatitis patients at a higher rate than that in the healthy control group. While Abulude O A *et al*. 2017 **[16]**, revealed that, among HBV patients, only 75% had elevated levels of AST .

The results of current study were in line with previous studies which showed significantly elevated serum concentration of ALT, AST, and TSB in hepatitis patient compared to healthy control **[17]**.

While, Saleh D A, 2015 **[18]**, found non-significant changes in the level of ALT, AST, and total bilirubin among control and hepatitis B patients group.

In the present study, the level of albumin concentration decrease in CHB patients. This finding was coinciding with Maulidia V N R *et al*.,2020 **[19]**, was

reporting that impaired albumin synthesis in hepatic cell, and chronic liver cell injuries lead to decrease serum albumin levels .

The results of the study revealed that Prothrombin time is prolonged in chronic hepatitis B patients compare to control subjects. The most important function of the liver is the synthesis of coagulation factors (except factor VIII). Prothrombin time evaluate the rate of converted prothrombin to thrombin and this represent the synthetic function of the liver. The main cause of prolong in prothrombin time are vitamin K deficiency, liver disease, warfarin therapy and consumptive coagulopathy **[11]**.

This study concomitant with Schmucker D L, 1998 **[20]**, they observed that several tests faile to recognize significant age-associated deficits in hepatic functions. The study of Tietz N W *et al.*, 1998 **[21]**, concluded that based on parameters such as hepatic enzyme profiles, and serum albumin, many liver functions were sustained in elderly subjects. Increasing age lead to decline bilirubin values, possibly reflect muscle mass reduction and decline hemoglobin concentration **[20]**, this explain the result of the present study**.** But our results contradicted the study of Cieslak K P *et al*.,2016 **[22]** ,shown that liver function declines with age.

Our study is in accordance with Adiga U S, 2016**[23]**, who suggest a low AST levels in women.

Cotler S *et al.*, 2010 **[24]**, found decreased ALT in female. With same living conditions, ALT level variation were noted in different genders **[25]**. This difference in liver enzymes is related to the hormones and muscle mass.

Rosenthal P *et al.*,1984 **[26]**, reported decreased bilirubin concentration in women. This might be endorsed to elevated conjugation rate and excretion of bilirubin in females because of the effect of gonads hormones.

Contradictory reports were given by Guattery J M *et al.*,1987 **[27]**, who found increase bilirubin concentration in women with biliary cirrhotic.

Our findings on serum albumin and PT are supported by Adiga U S, 2016 **[28]**, they reported low albumin levels and prolong PT in females might be due to increased degradation, this mean protein synthesis in female is lower than males.

Serum level of CK18 increase by necrosis of the liver canot reveal the lesion, which may be due to no active inflamatory lesion of the liver in patient with chronic liver disease, necrosis of hepatocytes is not the officer pathophysiological factor. CK18 levels were also relate to BCLC stage, suggesting that CK18 may be a valu able needle of disease severity**[29].**

CK18 is released into the blood from dead cell and its serum level thus represent the levels of apoptosis and necrosis. Serum level of total CK18 and CK18 cleavage fragments were increase in patient with liver cirhosis and HCC in contradiction of a back-ground of chronic hepatitis B, compare with a control group, histological examinaton of the liver reveale that serum CK-18 level correlate with the fibrosis stage, lobular inflammation, portal inflamation, steatosis, hepatocelular balooning **[30].**

Conclusion

Among chronic hepatitis B patient in Babylon province, abnormality of liver function tests in different age and gender groups; show the significant linke with the progression of chronic hepatitis B virus.

References:

[1] Schillie S, Vellozzi C, Reingold A, et al. Prevention of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices. MMWR Recomm Rep 2018;67(1):1–31.

[2] Wang G, Duan Z. Guidelines for Prevention and Treatment of Chronic Hepatitis B. J Clin Transl Hepatol. 2021; 9(5): 769–791.

[3] Rana Warid Maya. Analysis of some cytokines in patients infected with hepatitis b and c viruses. a thesis submitted to the council of the college of science/ University Of Thi-Qar . 2021

[4] Mauss S, Berg T, Rockstroh J,et al. Hepatology-A clinical textbook. 10th Ed. Frankfurt. Flying Publisher. 2020;418.

[5] Wiktor SZ. Hutin YJ. The global burden of viral hepatitis: better estimates to guide hepatitis elimination efforts. Lancet. 2016; 388(10049):1030- 1031.

[6] Schmit N ,Nayagam S, Thursz M R, et al. The global burden of chronic hepatitis B virus infection: comparison of country-level prevalence estimates from four research groups. International Journal of Epidemiology. 2021; 50(2): 560–569.

[7] Betharina N, Hendriyono FX, Mashuri. Perbedaan hasil laboraturium penderita hepatitis B dan C kronis dengan derajat fibrosis hati. Berkala Kedokteran. 2017; 13(1): 41-46.

[8] Kim J V& Wu GY. Body building and aminotransferase elevations: A review. J. Clinic.& Translational Hepato.2020; 8(2):161–167.

[9] Carr B, Guerra V. Serum albumin levels in relation to tumor indis in hepatocellular carcinoma patients. The International Journal of Biological Markers. 2017; 32(4): 391-396.

[10] Nickless G. How to interpret liver function tests. Clin. Pharma.2009; 1(9): 363–366.

[11] Hussein N, Shemran K A, Ali Z A, et al. The study of the effect of Helium –Neon Laser irradiation on the Albumin. Medical journal of Babylon. 2008;5(2): 34-42.

[12] Alaluf MB, Shlomai A. New therapies for chronic hepatitis B. Liver Int. 2016;36(6):775-82. Alaluf MB, Shlomai A. New therapies for chronic hepatitis B. Liver Int. 2016;36(6):775-82.

[13] Rutherford A, King LY, Hynan LS, et al. Development of an accurate index for predicting outcomes of patients with acute liver failure. Gastroenterology. 2012;143(5):1237 1243.

[14] 14-Ewadh M J, Badr S, Al Musawi I H, et al. Correlation of new vitamin C derivatives with alanine aminotransferase and aspartate aminotransferase activities. Medical journal of Babylon. 2014; 11(2): 13-21.

[15] Al-Haidary B, Abdul-Kareem S, Azziz F, et al. Viral Load Among the Sera of Iraqi Hepatitis C Virus Patients. J. Fac. of Med. Baghdad.2018; 49(4): 461-466.

[16] Abulude O A, Ahmed I, & Sadisu F U. Assessment of Hepatitis B Viral Infection as a Predictor of Hepatic Enzymes and Compounds Alteration among Antenatal Patients. Medical scie.2017; 5(4): 24-31.

[17] Saxena R. Practical Hepatic Pathology: A diagnostic approach e-book: A volume in the pattern recognition series. Elsevier Health Scie.2017; 212- 233.

[18] Saleh D A. Prevalence and risk factors of HBV infection among pregnant women in urban and rural Egyptian communities. J. Liver.2015; 4(3):1-9.

[19] Maulidia V N R , Wardhani P , Setyoboedi B. AST, ALT and Albumin Level in Chronic Hepatitis B Patients with and without Complications of Cirrhosis and Hepatocellular Carcinoma. Indonesian Journal of Clinical Pathology and Medical Laboratory. 2020; 26 (3) : 344 – 349.

[20] Schmucker D L. Aging and the Liver: An Update. Journal of Gerontology: BIOLOGICAL SCIENCES.1998; 53(5): 315-320.

[21] Tietz NW, Shuey DF, Wekstein DR. Laboratory values in fit aging

[22] Individuals sexagenarians through centenarians. Clin Chem. 1992;

[23] 38:1167-1185.

[24] Cieslak K P, Baur O, Verheij J, et al. Liver function declines with increased age. International Hepato-Pancreato-Biliary Association. 2016; 18: 691–696.

[25] Adiga U S. Gender Differences in Liver Function Tests in Coastal Karnataka. IOSR Journal of Dental and Medical Sciences. 2016; 15(8): 30-32.

[26] Cotler S, Dhamija M, Luc B,et al. Theprevalence and clinical correlates of elevated ALT levels in an urban Chinatowncommunity. Journal of Viral Hepatitis. 2010;17(2):148-152.

[27] Chen CH, Huang MH, Yang JC, et al. Prevalence and etiology of elevated serum alanine aminotransferase level in an adult population in Taiwan. Journal of gastroenterology and hepatology. 2007;22(9):1482-1489.

[28] Rosenthal P, Pincus M, Fink D. Sex- and Age-Related Differences in Bilirubin Concentrations in Serum. Clin chem.1984; 30(8): 1380-1382 .

[29] Guattery JM, Faloon WW. Effect of estradiol upon serum enzymes in primary biliary cinhosis. Hepatology .1987; 7: 737-742.

[30] Adiga U S. Gender Differences in Liver Function Tests in Coastal Karnataka. IOSR Journal of Dental and Medical Sciences. 2016; 15(8): 30-32.

[31] Ku NO, Strnad P, Bantel H, Omary MB. Keratins: Biomarkers and modulators of apoptotic and necrotic cell death in the liver. Hepatology. 2016;64(3):966 976.

[32] Bettermann K, Mehta AK, Hofer EM et al (2016) Keratin 18-deficiency results in steatohepatitis and liver tumors in old mice: a model of steatohepatitis-associated liver carcinogenesis. Oncotarget

[33] 7:73309–73322.