SERUM LEVELS OF B_2MICROGLOBULIN AND SOME BIOCHEMICAL PARAMETERS AMONG CHRONIC HEPATITIS B PATIENTS

Maysoon K. Al-Shaikle
Department of Chemistry, College of Science, Baghdad University.

Abstract

The aim of this study was to estimate the level of $\beta_2$microglobulin (B2M), total serum bilirubin (TSB) and alanine aminotransferase (ALT) in the sera of (30) chronic hepatitis B (CHB) patients (patients group) and (30) healthy HBV carriers as (control group). The study showed that levels of β_2 microglobulin were high among patients group (3.41 ± 1 mg/L), while it was normal among control group (1.42 ± 0.35 mg/L). Regarding biochemical parameters, it was found that (TSB) and (ALT) were increased among patients group, and their level were (37.66 ± 30.8 μmol/L) and (43.6 ± 32.51 U/L) respectively. While it was normal among control group (4 ± 0 μmol/L) and (17.4 ± 7.16 U/L) respectively.

Keywords : β2microglobulin, Chronic Hepatitis B.

Introduction

Viral hepatitis is a systemic disease primarily involving the liver as a main target for viral replication which characterized clinically by fever, jaundice and gastrointestinal symptoms [1]. Chronic HBV infection is usually defined as detectable hepatitis B surface antigenemia (HBsAg) for a period of six months or more [2]. Approximately (30%) of the world's population are infected with HBV worldwide, (360 million) of them suffer from chronic HBV infection resulting in over 520 000 deaths each year [3]. Chronic HBV infection appears to be the cause of (50% to 60%) of hepatocellular carcinoma (HCC) worldwide [4]. Iraq is among the intermediate HBV endemic countries, because the carrier rate of HBV is between (3%-4.5%) among normal population [5]. Lower carrier rate was detected in the last (10 years) among Iraqi blood donors (1%- 2%) [6]. β_2 microglobulin is an amino acid peptide component that increases in inflammatory conditions and when lymphocyte turnover increases [7]. β_2 microglobulin also known as B2M is a component of MHC class I molecules, which are present on all nucleated cells (but not red blood cells) [8]. B2M is necessary for cell surface expression of Major Histocompatibility Complex class I (MHC class I) and stability of the peptide binding groove. In fact, in the absence of B2M, very limited amounts of MHC class I (classical and non-classical) molecules can be detected on the surface. In the absence of MHC class I, CD8 T cells cannot develop [9]. Bilirubin (formerly referred to as hematoidin) is the yellow breakdown product of normal heme catabolism. Heme is found in hemoglobin, a principal component of red blood cells. It is responsible for the yellow color of bruises and the yellow discoloration in jaundice [10]. Daily production of unconjugated bilirubin is 250 to 350 mg, mainly from senescent erythrocytes [11]. Clearance at normal values is about 400 mg/day in adults [12]. The half-life of unconjugated bilirubin is (<5 minutes) [13]. Conjugated bilirubin is excreted into bile and is essentially absent from blood in normal individuals. Delta bilirubin (also sometimes termed biliprotein) is produced by reaction of conjugated bilirubin with albumin [14]; it has a half-life of about (17-20 days, the same as albumin), accounting for prolonged jaundice in patients recovering from hepatitis or obstruction [15]. Alanine aminotransferase (ALT, also sometimes termed GPT or SGPT) are widely distributed in cells throughout the body, it is found primarily in liver and kidney, with lesser amounts in heart and skeletal muscle. ALT activity in liver is about (3,000 times) serum activities[16]. ALT is exclusively cytoplasmic [17]. The half-life of total ALT is (47 ± 10 hours) [18]. The aim of this study was to estimate the level of $\beta_2$microglobulin (B2M), total serum bilirubin (TSB) and alanine aminotransferase (ALT) in

the sera of (30) chronic hepatitis B (CHB) patients (patients group) and (30) healthy HBV carriers as (control group).

Materials and Methods

The study was conducted in The Ministry of Health/ Central Public Health Laboratories/ Biochemistry Referral Laboratory. Thirty Chronic hepatitis B (CHB) patients (patients group) and thirty healthy HBV carriers (control group), age between (20-49 years) for both, patients and control groups. From each subject included in this study, five to ten ml of blood was collected by vein puncture using disposable syringes. The blood was placed in plastic disposable plain tubes, and allowed to clot at room temperature and serum was separated by centrifugation at 1500xg for 5 min, then stored and frozen at –20^{o}C [19]. Serum and all reagents were allowed to stand at room temperature before use in the test. Estimation of $\beta_2$microglobulin were carried by VIDAS $\beta_2$microglobulin (B2M) kit (BioMerieux, France) which is a quantitative test for use on the (miniVIDAS) analyzer using the Enzyme Linked Fluorescent Assay technique (ELFA). The assay principle combines a two-step enzyme immunoassay sandwich method with a final fluorescent detection. Total serum bilirubin (TSB) determined by applying a modified method of Jendrassik and Grof (1938) [20]. Serum alanine aminotransferase (ALT) estimated according to the colorimetric method of Reitman and Frankel (1957) [21].

Statistical Analysis

Results for estimation the level of (B2M), (TSB) and (ALT) were analyzed statistically. Values were expressed as a (mean ± SD). The level of significance was determined by student's t-test when ($P<0.05$) [22].

Results and Discussions

Distribution of CHB patients according to their age is shown in Table (1). It was found that most of patients (43.4 %) were located within age range between 40-49 years. This result in agreement with the previous studies done in Iraq as Youssif (1998) [23] who found the mean age was (42y) and Al-Hilli and Ghadhban (2000) [24] who reported the most common age group for hepatitis B was the fourth decade, also several studies in the world agreed with this study results as Dienstag *et al*., (1995) [25] who reported (42.6y) the mean age for CHB. Table (2) summarized the levels of (B2M), (TSB) and (ALT) in sera of CHB patients and healthy HBV carriers. The level of $\beta_2$microglobulin was higher among CHB patients (3.41 ± 1 mg/L) than carriers group (1.42 ± 0.35 mg/L) with significant correlation ($P<0.001$). These results were in agreement with Meral Akdogan *et al*, whom found that serum $\beta_2$microglobulin levels were significantly higher among CHB patients [26], while Fabíola and Liliete (2005) [27] found that the range of (B2M) among Brazilian blood donors was (1.3-3.9 mg/L) and the mean was (2.46 mg/L). It was found that the level of TSB among chronic group was higher than carriers group which was (37.66 ± 30.8 μmol/L) and (4 ± 0 μmol/L) respectively with significant correlation ($P<0.001$). This result is in agreement with Youssif (1998) [23] and Sabri (2003) [28]. Regarding ALT levels it was found that the mean level among chronic group (43.60 ± 32.51 U/L) which was higher than carrier group (17.4 ± 7.16 U/L) and the difference was significant ($P<0.001$). These differences may be due to the higher level of inflammation of hepatocytes among CHB patients which could be more than healthy HBV carriers. The increase of ALT enzyme level strongly suggests hepatocellular injury [29]. Patients and controls divided into (3) groups according to age (20-29 y), (30-39 y) and (40-49 y). Concentration of (B2M) increased with age as shown in Table (3) and Fig.(1), mean values in patient group were (2.15 ± 0.54 mg/L), (3.08 ± 0.18 mg/L) and (4.25 ± 0.66 mg/L) respectively, while in control group the mean values were (1.38 ± 0.43 mg/L), (1.45 ± 0.27 mg/L) and (1.51 ± 0.2 mg/L) respectively and the difference were significant ($P<0.001$), ($P<0.001$) and ($P<0.001$) respectively. Table (4) and figure (2) show the levels of (TSB) according to age groups, the concentration increased among patient group and the mean values were (24.60 ± 24.3 μmol/L), (32.92 ± 23.80 μmol/L) and (34.48 ± 28.39 μmol/L) respectively, while in control group the levels were unchanged and within normal value (4± 0 μmol/L). Regarding

(ALT) the levels were increased with age in patient group only, as shown in Table (5) and figure (3), their mean values were (24.66 ± 15.80 U/L), (39.18 ± 30.59 U/L) and (51.76 ± 30.62 U/L) respectively, while in control group the levels were (19.11 ± 8.45 U/L), (14.25 ± 5.28 U/L) and (16.60 ± 2.07 U/L) respectively. Results of correlation studies between these Parameters in sera of Chronic Hepatitis B Patients revealed that there were positive relationship between age groups & B2M and between age groups & ALT and between age groups & TSB respectively as shown in Figs. (4, 5 & 6).

Table (1)
Distribution of CHB patients according to their age.

Age groups (Years)	*No.*	*%*
20-29	6	20
30-39	11	36.6
40-49	13	43.4
Total	30	100

Table (2)
Serum (B2M), (TSB) and (ALT) levels of CHB patients and HBV healthy carriers.

Parameter	*CHB Patients Mean±SD*	*Control Mean±SD*	*P value*
B2M	3.41 ± 1	1.42 ± 0.35	p<0.001
TSB	37.6 ± 30.8	4 ± 0	p<0.001
ALT	43.6 ± 32.51	17.4 ± 7.16	p<0.001

Table (3)
Serum B2M level of CHB patients and HBV healthy carriers regarding the age groups.

B2M < 2.0 mg/L [7]			
Age groups	*CHB Patients Mean±SD*	*Control Mean±SD*	*P value*
20-29	2.15 ± 0.54	1.38 ± 0.43	p<0.01
30-39	3.08 ± 0.18	1.45 ± 0.27	p<0.001
40-49	4.25 ± 0.66	1.51 ± 0.20	p<0.001

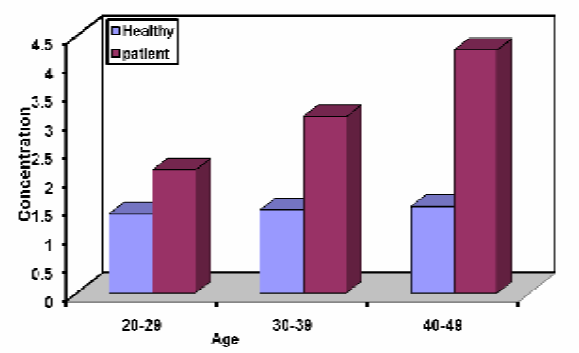

Fig. (1): Comparison of serum B2M level between CHB patients and HBV healthy carriers regarding the age groups.

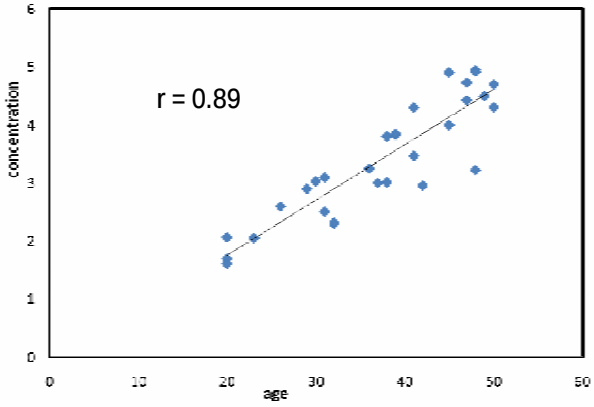

Fig. (4): Correlation of serum B2M level between CHB patients and HBV healthy carriers regarding the age groups.

Table (4)
Serum TSB level of CHB patients and HBV healthy carriers regarding the age groups.

TSB ≤5.1–20 µmol/L[30]			
Age groups	*CHB Patients Mean±SD*	*control Mean±SD*	*P value*
20-29	24.60 ± 24.30	4 ± 0	0.5
30-39	32.92 ± 23.80	4 ± 0	p>0.001
40-49	34.48 ± 28.39	4 ± 0	p<0.01

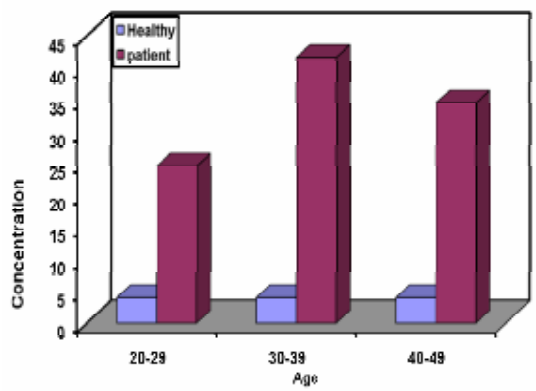

Fig. (2): Comparison of serum TSB level between CHB patients and HBV healthy carriers regarding the age groups.

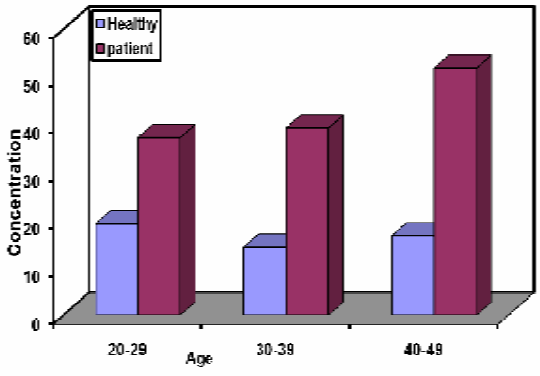

Fig. (3): Comparison of serum ALT level between CHB patients and HBV healthy carriers regarding the age groups.

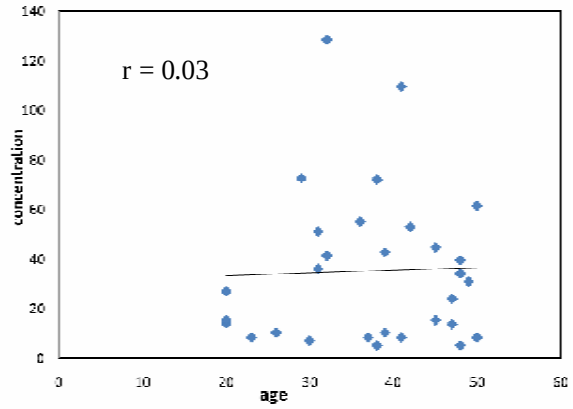

Fig. (5): Correlation of serum TSB level between CHB patients and HBV healthy carriers regarding the age groups.

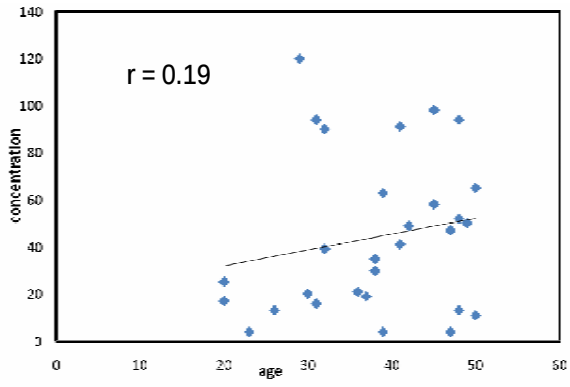

Fig. (6): Correlation of serum ALT level between CHB patients and HBV healthy carriers regarding the age groups.

Table (5)
Serum ALT level of CHB patients and HBV healthy carriers regarding the age groups.

ALT 0-35 U/L [31]			
Age groups	*CHB Patients Mean±SD*	*control Mean±SD*	*P value*
20-29	24.66 ± 15.80	19.11 ± 8.45	0.5
30-39	39.18 ± 30.59	14.25 ± 5.28	0.2
40-49	51.76 ± 30.62	16.60 ± 2.07	0.02

References

[1] Jawetz, Melnick and Adelberg's. 'Hepatitis viruses' In: *Medical Microbiology*. Brooks GF, Butel JS and Morse SA (eds.), 24th ed., Appleton and Lange(2007).

[2] Hyams KC. Risks of chronicity following acute hepatitis B virus infection: A review. *Clin Infect Dis* ; 20: (1995), 992-1000.

[3] Yan Wang, Lai Wei, Dong Jiang, Xu Cong, Ran Fei, Jiang Xiao, Yu Wang. *In vitro* resistance to interferon of hepatitis B virus with precore mutation. *World J Gastroenterol*; 11,5, (2005), 649-655.

[4] Hayashi PH, Di Bisceglie AM. The progression of hepatitis B and Cinfections to chronic liver disease and hepatocellular carcinoma: epidemiology and pathogenesis. Med Clin North Am; 89,2, (2005), 371-389.

[5] Omer AR and Al-Douri S. Viral hepatitis in Iraqi normal population. Proceedings of the 6th International congress of virology, Sendai- Japan, (1984), p 32-36.

[6] Omer AR. Viral hepatitis among hemophiliac and thalassemic patients. (2004)CDC/Iraq.

[7] Cynthia C. Chernecky and Barbara J. Berger. Laboratory Tests and Diagnostic Procedures, 5th ed., Saunders an imprint of Elsevier Inc (2008).

[8] Güssow D, Rein R, Ginjaar I, Hochstenbach F, Seemann G, Kottman A, Ploegh HL. "The human beta 2-microglobulin gene. Primary structure and definition of the transcriptional unit". *J. Immunol.*; 139 ,9, 1987,pp 3132–8. PMID 3312414.

[9] "Definition of beta-2-microglobulin - NCI Dictionary of Cancer Terms".

[10] Golonka, Debby. "Digestive Disorders HealthCenter: Bilirubin". WebMD.pp.3. http://www.webmd.com/digestive-disorders/Bilirubin-15434page. Retrieved 2008-11-19.

[11] Chowdhury JR, Wolkoff AW, Chowdhury NR, Arias IM. Hereditary jaundice and disorders of bilirubin metabolism. In: The metabolic and molecular bases of inherited disease, Scriver CR, Beaudet AL, Sly WS, Valle D, Stanbury JB, Wyngaarden JB, Fredrickson DS, eds., New York, McGraw-Hill, Inc.; (1995) 2:2161-2208.

[12] Berk PD, Martin JF, Blaschke TF, Scharchmidt BF, Plotz PH. Unconjugated hyperbilirubinemia: physiologic evaluation and experimental approaches to therapy. Ann Intern Med 1975;82:552-570.

[13] Bloomer JR, Berk PD, Vergalla J, Berlin NI. Influence of albumin on the extravascular distribution of unconjugated bilirubin. Clin Sci Mol Med 1973; 45:517-521.

[14] McDonagh AF, Palma AA, Lauff JJ, Wu TW. Origin of mammalian biliprotein and rearrangement of bilirubin glucuronides in vivo in the rat. J Clin Invest . 74: (1984)763-770.

[15] Fevery J, Blanckaert N. What can we learn from analysis of serum bilirubin. J Hepatol; 2,1986,113-121.

[16] Lott JA, Wolf PL. Alanine and aspartate aminotransferase (ALT and AST). Clinical enzymology: a case-oriented approach. Chicago, Year Book Medical Publishers, 1986; 111-138.

[17] Rej R. Measurement of aminotransferases: Part 1. Aspartate aminotransferase. CRC Crit Rev Clin Lab Sci 1984; 21:99-106.

[18] Price CP, Alberti KGMM. Biochemical Assessment of Liver Function. in Wright R, Alberti KGMM, Karran S, Millward-Sadler GH (eds.): Liver and Biliary Disease–Pathophysiology, Diagnosis, Management. London: W.B. Saunders, 1979, 381-416.

[19] Stewart,C. E., Koepke, J. A.. Basic quality assurance practices for clinical laboratories. In Howanitz , J. F. (eds). Laboratory quality assurance. 4th ed. P : (1987),217 . J. B. Lippincott, Philadelphia.

[20] Jendrassik L. and Grof P. *Biochem. Z. 1938*; 297: 81.OR Jendrassik L. and Grof P (1938). Vereinfachte photometrische methode zur bestimmung des bilirubins. Biochem Z 1938; 297:81–9.

[21] Reitman S and Frankel S. Estimation of aminotransferases enzyme in the blood. *Am J Clin. Pathology* 1957; 28:56.

[22] Kaplan A and Pasce AJ. Clinical Chemistry, Theory, Analysis, Correlation, 2nd Ed.; (1989)pp. 772 the C.V. Mosby Company.

[23] Youssif TH. Immunological study of patients with chronic active hepatitis B. Ph.D. thesis 1998, College of Medicine, University of Baghdad.

[24] Al-Hilli HAA and Ghadhban JM. Prevalence of serological markers of HBs Ag and HCV antibodies among blood donors and certain risk groups. *J Fac Med. Baghdad*; 42,1,2000,45.

[25] Dienstag JL, Perrillo RP, Schiff ER. A preliminary trial of lamivudine for chronic hepatitis B infection. *N. Engl. J. Med.*; 333, 25, 1995, 1657.

($\beta_2$microglobulin) أرتفاعـــا بـــين المرضـــى (1±3,41 mg/L) وكانت طبيعية لدى مجموعة الحـاملين الصحاء (0,35±1,42 mg/L) في حين أبدت الفحوصـات الكيموحيوية (اليرقـان TSB) و (خميـرة الكبـد ALT) ارتفاعا ملحوظا لدى المرضى (30,8 ± 37,6 µmol/L) و (32,5 ± 43,6 U/L) على التوالي في حين كان طبيعية لدى مجموعة الحـاملين الاصـحاء (0 ± 4 µmol/L) و (7,1 ± 17,4 U/L) على التوالي.

[26] Meral Akdogan *et al*. Acute exacerbation during interferon alfa treatment of chronic hepatitis B: frequency and relation to serum beta-2 microglobulin levels. *Journal of Gastroenterology* 38, 5, 2003, pp 465-470.

[27] Fabíola Branco Filippin and Liliete Canes Souza Serum β_2 microglobulin values among healthy Brazilians using a DPC IMMULITE® assay, Clinics vol.60 no.1, 2005, São Paulo Jan. /Feb. .

[28] Sabri HJA. The diagnostic role of liver biopsy in grading, staging, and etiology of chronic hepatitis. A Thesis fellow ship 2003. Iraqi Commission for Medical Specialization in pathology.

[29] Berenguer M and Wright T L Viral hepatitis. *In*: Sleisenger and Fordtran's Castrointestinal and liver disease, pathophysiology / diagnosis / management. Edited by Feldman M, Friedman L S and Sleisenger M H, Vol 3. 2002. PP 1278.

[30] Fischbach, Frances Talaska; Dunning, Marshall Barnett. A Manual of Laboratory and Diagnostic Tests, 8th ed., 2009, pp: 20, Lippincott Williams & Wilkins.

[31] Diana Nicoll, Stephen J. McPhee, Michael Pignone, William M. Detmer and Tony M. Chou. Pocket guide to diagnostic tests. 3rd ed., 2001. pp: 57. New Yourk, Lange Medical Books/ McGraw-Hill.

الخلاصة

تهدف هذه الدراسة إلى تحديد مستويات الوسمة المناعية ($\beta_2$microglobulin) (B2M) وبعـــض الفحوصـــات الكيموحيوية (اليرقان TSB) و (خميرة الكبد ALT). وقد شملت الدراسة (30) مريضا عراقياً مصابا بالتهاب الكبـد الفيروسي المزمن نـوع (ب) (CHB patients) وكـذلك علـــى (30) شخـــصا مـــن الحـــاملين ألأصـــحاء (Healthy HBsAg Carriers) للمستـــضد الـــسطحي لفيروس التهاب الكبد نوع (ب). كـان المرضـى الـذين تتراوح أعمارهم بين (40 الى 49) سـنة يـشكلون نـسبة (43,4%). لقد أظهرت النتائج لمستوى الوسـمة المناعيـة