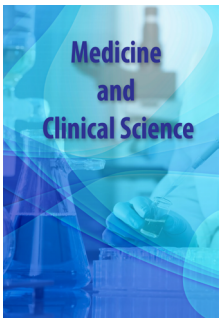
Medicine & Clinical Science

Original Article



Incidences of Hepatitis-B Within Kirkuk Province in Iraq, a Biochemical Study of Some Liver Enzymes

Ayoub A Bazzaz[1*], Ahmad S Ahmad[2], Tara N Azghar[2] and Noorhan A Chelebi[3]

[1]*Department of Basic Sciences, Faculty of Dentistry, University of Al-Kitab, Altin Kopri, Kerkuk, Iraq*
[2]*Department of Laboratory Technology, Faculty of Medical Technology, University of Al-Kitab, Altin Kopri, Kerkuk, Iraq*
[3]*Department of Chemistry, Faculty of Education for Pure Sciences, University of Kerkuk, Iraq*

Correspondence

Ayoub A Bazzaz

Department of Basic Sciences, Faculty of Dentistry, University of Al-Kitab, Altin Kopri, Kerkuk, Iraq

E-mail: ayoub.a.bazzaz@uoal-kitab.edu.iq

- Received Date: 01 Aug 2023
- Accepted Date: 12 Aug 2023
- Publication Date: 17 Aug 2023

Keywords

Enzymes; Hepatitis-B; ALT, ALP, AST, GGT, TSB, Kirkuk, Iraq.

Abstract

Geographical distribution of Hepatitis-B, both acute and chronic caused by the hepatitis-B virus (HBV) has always been attracting attention worldwide. Prevalence of HBV does vary among genders and ages while symptomatic and asymptomatic are not uncommon. The present research involved assessment of three different parameters i.e. four liver enzymes, hematological and serological factors in 91 patients (70 male and 21 female) compared with 28 control counterpart (23 male and 5 female) admitted a private Medical laboratory in Kirkuk province. The arithmetic values of the 4 main liver enzymes, Alanine Trans-Aminase (ALT), Alkaline phosphatase (ALP); aspartate transaminase (AST) and Gamma-Glutamyl transferase (GGT) showed significant increase (p≤0.05-0.001) in Hepatitis-B patients in comparison with their counterpart healthy individuals. An insignificant differences was detectable in both WBC and Hb% values meanwhile significant increases (20-25 folds) elevation was detected in the total serum bilirubin (TSB) of HBV patients. Variations in the values of these enzymes, particularly ALT enzyme, was also prominent between the genders e.g. in male patients were comparable with their counterparts female. The values of the enzyme ALT, particularly in female hepatitis, were four folds higher than males; however, other enzyme showed lesser value variations ranged between significant to insignificant. It is conclude that regular check-up of these enzymes deems necessary for personal health purposes of HBV patients.

Introduction

Hepatitis, a general term that refers to inflammation of the liver caused by a variety of different viruses i.e. hepatitis A, B, C, D and E is a homologous serum jaundice [1,2]. The Hepatitis-B, particularly, is a serious but common infectious disease of the liver, affects millions of people worldwide caused by the Hepatitis-B virus (HBV) that lead to both acute and chronic infection [3], meanwhile, deaths resulting from acute stage HBV infections, yet, are rare [4]. Initial infection of HBV, could be asymptomatic while others might be symptomatic that appear within 30-180 days following infection. Symptoms, may include a rapid onset of sickness with nausea, vomiting, yellowish skin, fatigue, dark urine, and abdominal pain [5]. The diagnosis is usually confirmed by testing the blood for parts of the virus and/or for antibodies against the virus [6]. Transmission of the virus occur either via intravenous drug use, sexual intercourse, working in healthcare, blood transfusions, dialysis, tattooing, living with an infected person, travel in countries with high infection rates, and living in an institution [7]. Cirrhosis and liver cancer are responsible for most HBV-related deaths [8]. Since 1982, the HBV infection has been preventable by vaccination while the vaccine of hepatitis-B had between 98% and 100% impact in preventing infection by 2022 [8]. In 2019, an estimated 296 million people (3.8%) of the global population, had chronic hepatitis-B infections while another 1.5 million developed acute infections that year, with a total of 820,000 deaths occurred as a result of HBV [9]. The disease is most prevalent in Africa (affecting 7.5% of the continent's population) and in the Western Pacific region (5.9%) [10]. Infection rates are 1.5% in Europe and 0.5% in the Americas and is estimated that, about a 30% of the world's population have been infected with HBV at one point in their lives [8,9]. The virus interferes with the functions of the liver while replicating in hepatocytes. The immune system is then activated to produce a specific reaction to combat and possibly eradicate the infectious agent. As a consequence of pathological damage establishes and the liver becomes inflamed. HBV may be the cause of up to 80% of all cases of hepatocellular carcinoma worldwide, second only to tobacco among known human carcinogens [11]. HBV transmitted through percutaneous or parenteral contact with infected blood, body fluids, other viruses, are larger and cannot cross the placenta

Citation: Chelebi NA, Al-Ani AB, Ali SJ, Bazzaz AA, Al-Basheer NT. Can Leukoerythroblastic Activation Indicate Coronavirus Infection? Med Clin Sci. 2023;5(6):1-5

to infect the fetus unless via maternal-fetal broken barrier e.g. via amniocentesis [7]. Infected pregnant women with HBV can, yet, transmit the disease to their babies at birth. Whenever, babies are not vaccinated at birth, many of them may develop lifelong HBV infections, others may develop liver failure or liver cancer later in life. Sexual intercourse with multiple partners or with persons who have multiple partners can be dangerous [9].

National programs had made the hepatitis-B vaccine available for infants in 190 countries by the end of 2021 [12]. To further prevent infection, the WHO recommends testing all donated blood for hepatitis-B before using it for transfusion. Using antiviral prophylaxis to prevent mother-to-child transmission is also recommended, as is following safe sex practices, including the use of condoms [13]. In 2016, the WHO set a goal of eliminating viral hepatitis as a threat to global public health by 2030. Achieving this goal would require the development of therapeutic treatments to cure chronic hepatitis-B, as well as preventing its transmission and using vaccines to prevent new infections [14].

Since the development of jaundice, the characteristic feature of liver diseases, a correct diagnosis can only be made by testing patients' sera for the presence of specific anti-viral antigens or antibodies. Of the many viral causes of human hepatitis few are of greater global importance than HBV. The severe pathological consequences of persistent HBV infections include the development of chronic hepatic insufficiency, cirrhosis, and hepatocellular carcinoma (HCC). Plus, infection occurs very often in early childhood when it is asymptomatic and often leads to the chronic carrier state. More than 2,000 million people alive today have been infected with HBV at some time in their lives. Of these, about 350 million remain infected chronically and become carriers of the virus [15]. According to WHO reports, 75% of the world's population live in areas where there are high levels of infection. Every year there are over 4 million acute clinical cases of HBV, and about 25% of carriers, 1 million people a year, die from chronic active hepatitis, cirrhosis or primary liver cancer.

Biochemical and serological studies on hepatitis-B in Iraq are scanty and almost local e.g. within the hospitals and private laboratories. The present research provides biochemical and serological data on hepatitis-B victims, from both genders, admitted private laboratories for checkup or treatment in Kerkuk city, Iraq.

Materials and methods

This research has involved analysis of the biochemical parameters of 91 patients (70 male and 21 female) and their counterparts of 28 control healthy individuals (21 male and 5 female) residents in Kirkuk province. Three mL blood samples from people infected with hepatitis-B virus (HBV) were collected from the peripheral blood for assay and analysed using apple spectrophotometer. Enzymatic assays were performed using kits Roche-Germany COBAS C35. The blood samples were separated using centrifuge at a speed 3,500/minute. Only 1.5 mL from serum samples were added on to the kit of COBAS C35. Serum samples were incubated for 5 minutes inside the system and the results were collected in 5 minutes. The enzymes (ALP, ALT, AST, GGT and TSB) are selected using the knobs of the system itself and five samples of the sera were placed in for each enzyme. By the spectrophotometer, the values of the enzyme are determined upon the amount of colour absorbed i.e. the lighter the colour the low values and the darker the higher values. These values are linked with printer and monitor where they could be both read and printed.

Assay of enzyme levels

An *in vitro* test for the quantitative determination of alanine aminotransferase (ALT) in human serum on Roche/Hitachi COBASc systems was done. ALT catalyses the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD+. The rate of NADH oxidation is directly proportional to the catalytic ALT activity which was determined by measuring the decrease in absorbance. The ALT Test included R1 ALT, IFCC. Reagent 1,2-Oxoglutarate 15 mmol/L, L-Alanine 500 mmol/L; LDH > 600 Ul/L; NADH <0.18 mmol/L; Tris Buffer 100 mmol/L; pH, 7.50±0.1, at 30°C. Bio-statistical methods using SPSS software was adopted to obtaine the arithmetic means, standard deviation (±sd) as well as p-values to demonstrate the differences between the parameters of hepatitis-B patients and control. Significant differences ranged ($p \leq 0.05$-0.001) and insignificance (NS) elevation ($p \geq 0.05$) are used to denote the degree of difference in the changed levels of parameters.

Table 1. *The arithmetic means, standard deviation (±sd) and p-values show the differences between the parameters of hepatitis-B patients and control analyzed using SPSS software. Stars refer to denote the significant differences ($p \leq 0.05$-0.001) and (NS) denotes insignificance elevation ($p \geq 0.05$).*

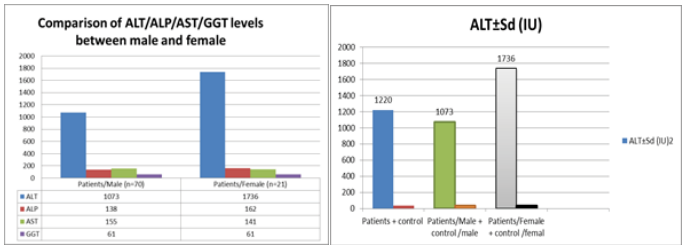
Samples/No. & T-Test	ALT±Sd (IU)	ALP± Sd (IU)	AST±Sd (IU)	GGT±Sd (IU)
Patients (n=91)	±313 1220	143±70	152±80	60±16
Control (n=28)	±9.336	±31.894	±11.429	37±13.6
Probability test ($p \leq 0.05$)	0.028*	0.00026**	0.0257*	0.010*
Patients/Male (n=70)	±1411073	±70 138	±85 155	61±15.7
Control/Male (n=23)	9.7 36±	±27.3 92	±1025	±1335
Probability test ($p \leq 0.05$)	0.0116	0.0014**	0.006**	0.035*
Patients/Female (n=21)	1736±728	162±71	141±58.1	61±17.5
Control/Female (n=5)	37±7.6	104±51	41±2.3	45±15.6
Probability test ($p \leq 0.05$)	0.021*	0.050*	0.00057**	0.0014*

Results

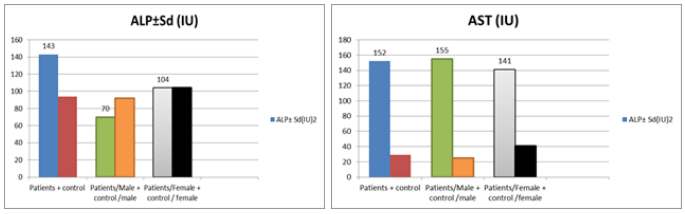
Ages of the patients, from both genders, admitted private laboratories for checkup appeared wide and extended between 18-89 years old. Almost all showed late stage of infection due to the values of the enzymes being measured.

Serological tests

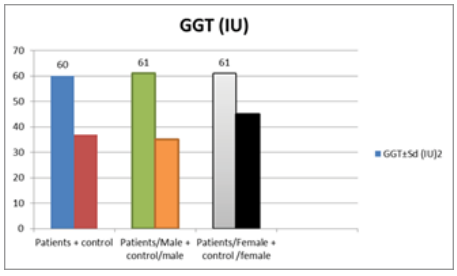
The arithmetic mean values of the liver enzymes in total 91 hepatitis-B patients: Alanine Trans-Aminase (ALT), Alkaline phosphatase (ALP); aspartate transaminase (AST) and Gamma-Glutamyl transferase (GGT) as well as total serum bilirubin showed dramatic and significant ($p \leq 0.05$-0.001) elevation in Hepatitis-B patients in comparison with their counterpart control or healthy individuals ranged from 2-24 folds with the highest in ALT (Table-1). Such significant increases were also prominent between genders with the highest in ALT of female (47 folds). The values of the enzyme ALT, particularly in female hepatitis, were nearly twice higher than males ranged between insignificant to highly significant in AST enzymes. Other enzymes, ALP, AST and GGT have displayed almost twice elevation (Figs 1-5).



Figures 1&2. Elevated levels of 4 enzymes in the HBV patients. Note the significant difference between the two genders regarding the ALT enzyme and comparison of ALT level between male and female patient.



Figures 1&2. Elevated levels of 4 enzymes in the HBV patients. Note the significant difference between the two genders regarding the ALT enzyme and comparison of ALT level between male and female patient.



Figures 5. Comparison of GGT level between male and female patient.

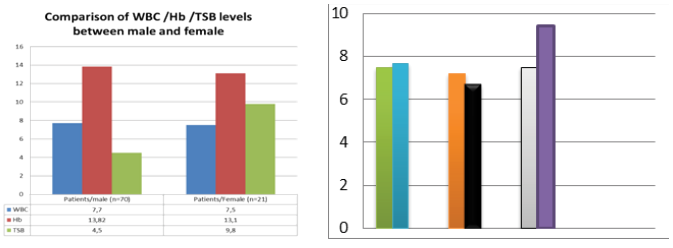
Using spectrophotometer specific the values of total serum bilirubin (TSB) in (mg/dL) showed significantly (14 folds) general increase ($p \leq 0.003$) as well as between male (11 folds) and female patients (25 folds) with ($p \leq 0.014$) in comparison with their counter parts, respectively (Fig. 6 and 7).

Blood parameters

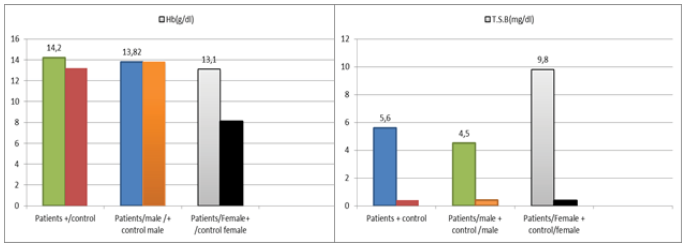
A significant increase ($p \leq 0.01$-0.032) in the rate of WBC in both gender patients was detectable (7.5×10^9/dL) in comparison with their control counterparts (7.2×10^9/dL), respectively. A a mild significant increase in the WBC in male patients (7.7×10^9/dL) vs. (6.7×10^9/dL) meanwhile, an insignificant differences was noticed in female patients (7.5×10^9/L) vs (5×10^9/L), respectively (Table-2). The percentage of Hb% had slightly, but significantly elevated in the total patients (both genders) (14.2±3 g/dL vs 13.2±2.2) in control while no differences were detectable when considered separately (Figs 8-9).

Table 2. *The arithmetic means, standard deviation (±sd) and p-values show the differences between the parameters of hepatitis-B patients and control analyzed using SPSS software. Stars denote the significant differences ($p \leq 0.05$-0.001) and (NS) denotes to insignificance ($p \geq 0.05$).*

Cases	WBC(10^9/L)	Hb (g/dl)	TSB (mg/dl)
Patients (n=91)	7.5 ± 4	14.2±3	5.6±1.0
Control (n=28)	7.2 ± 2	13.2±2.2	0.4±0.3
Probability test ($p \leq 0.05$)	0.03*	0.017*	0.0003**
Patients/male (n=70)	7.7 ± 5	13.82±2.2	4.5±1.9
Control/male (n=23)	6.7 ± 6	13.8±2	0.4±0.3
Probability test ($p \leq 0.05$)	0.01*	(NS)	0.014*
Patients/Female (n=21)	7.5±3	13.1±4	9.8±3
Control/Female (n=5)	7.4±2.1	12.1±2	0.38±0.33
Probability test ($p \leq 0.05$)	(NS)	(NS)	0.029*



Figures 6&7. The values of WBC, Hb% and TBS of both genders, male and female. Note the significant increase in the TSB values in female compared to males and comparison of WBC level between male and female patient and control.



Figures 8&9. Comparison of Hb% level between male, female patient and control and of TSB level between male, female patient and control.

Discussion

Generally, the stability in health is linked to the homeostasis of body organs including all enzymes i.e. liver kidney, adrenal and others. These enzymes may punctuate up and down according to status of homeostasis although not always [16]. Biochemical methods represents the most useful ways in assessing the impact of various diseases in human. Chronic infection with HBV, may sometimes be asymptomatic leading to cirrhosis over a period of several years. Hence, 'no apparent symptoms' status does not mean a healthy status of an individual but rather a dormant stage, which could announce, at later stages, leading to symptomatic [17]. In general, infection with HBV dramatically increases the incidence of hepatocellular carcinoma (HCC) which represent an approximately 50% of general hepatocellular carcinoma cases [18,19].

It has also been noted that rate of prevalence varies from country to another pending on various factors. In moderate prevalence areas where 2-7% of the population is chronically infected, the disease is predominantly spread horizontally, often among children, or vertically [20]. China's HBV infection rate is classified the highest, with an infection rate of 6.89%, of the moderate prevalence as of 2019 [21]. In India, the HBV prevalence is also moderate, i.e. between 2-4% [22]. However, other countries with low HBV prevalence include Australia (0.9%) [23], and those in the European Region with an average 1.5% [9]; and most countries in North and South America have an average of 0.28% [24]. Such variation in the prevalence may imply other factors i.e. gene variations, environmental, social, cultural and health education. The function of ALT in converting alanine into pyruvate, for cellular energy production [12] with a normal range (7-55 IU/L) is one of main liver enzyme that mainly found mainly in liver, and perhaps in other parts of human body. The ALT blood test is often included in both, liver and comprehensive metabolic panel, where healthcare providers in the Western countries and state hospitals in the middle eastern countries consider to assess the liver health. The importance of enzymatic analysis, therefore, represents the pre-diagnostic tool to assess the liver health condition and to measure the impact condition e.g. damage of the HBV to hepatocytes. The ALT leaks from the hepatocytes when infected [17].The ALT, particularly, is commonly used as a way of screening for liver disorders but other expected interpretation has also been valid. The high levels of ALT in the blood, found in this project, may indicate some damage to the liver and/or a liver condition. To confirm such a conclusion histological methods are required to study the architecture of the hepatocytes and carry out further biochemical and immunological tests. Other etiology of high ALT include obesity, anorexia, biliary disease, muscle damage and disease, heart attack, hypothyroidism, and infections and diseases that can impair liver function [25]. Alcohol breakdown produces free radical which damage hepatocytes and increase levels of inflammatory markers [26]. Excessive alcohol consumption leads to death of hepatocytes indicated by elevated levels of ALT ranges of ALT values showed reference specificity in patients (0-40 IU/L) [27,28]. Etiology of HBV cases of this project are not studied due to some social reasons as patients were reluctant to disclose private information to GPs or researchers. Most of these patients were smoker and had other diseases too. A very few patients have died during the course of study. In human, the test results should always be interpreted using the reference range from the laboratory that produced the result as well as gender difference [29].

Bilirubin, is an orange-yellow pigment that occurs normally when part of the RBC breaks down. The liver takes the bilirubin from the blood and changes its chemical make-up so that most of it is passed through as bile. Elevation of the bilirubin levels attributes to either the RBC are breaking down at an unusual rate or that liver is not breaking down waste properly and clearing the bilirubin from the blood [30]. Another option is presence of a problem somewhere along the pathway that transports the bilirubin from liver into the stool. In children and adults, doctors use it to diagnose and monitor liver and bile duct diseases which may refer to the possibility of cirrhosis, hepatitis, and gallstones. It'll also help determine the sickle cell disease or other conditions that cause hemolytic anemia. High levels of bilirubin can cause a yellowing of the skin and eyes, a condition doctors call jaundice. The bilirubin test involves measurement of amount of bilirubin in the blood and is used to help find the cause of health conditions like jaundice, anemia, and liver disease. In this project the range of a total serum bilirubin (TSB) elevation ranged between 11-25 folds in comparison with control. The elevation in TSB clearly confirms the liver disorder in HBV patients e.g. cirrhosis or even anemia which are not checked in this report. A regular health check-up is recommended to everybody in the society to conduct for personal and public security. The CBC is also recommended to check the blood parameters which could well be affected when liver enzymes are elevated.

Conclusion

It is concluded that ALT, GGT and TSB are elevated during chronic or/and severe hepatitis-B infection to indicate an unhealthy condition of liver where each individual should regularly have a check up on. The HBV is irrelevant to ages nor to gender and nobody is safe of its impact. There is no conflict of interest neither between other parties nor among the co-authors.

References

1. Logan CM, Rice MK. Logan's Medical and Scientific Abbreviations. J. B. Lippincott and Company, Philadelphia, United States 1987;pp. 232.
2. Barker LF, Shulman NR, Murray R, et al. Transmission of serum hepatitis. 1970. JAMA. 1996;276(10):841-844.
3. Chang MH. Hepatitis B virus infection. Semin Fetal Neonatal Med. 2007;12(3):160-167.
4. Thomas HC. Viral Hepatitis (4th ed.). Hoboken: Wiley; 2013; p. 83.
5. Rubin R, Strayer DS. Rubin's Pathology : clinic-pathologic foundations of medicine (5th ed.). Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins. 2008; p. 638.
6. Pungpapong S, Kim WR, Poterucha JJ. Natural history of hepatitis B virus infection: an update for clinicians. Mayo Clin Proc. 2007;82(8):967-975.
7. Cox AL, El-Sayed MH, Kao JH, et al. Progress towards

elimination goals for viral hepatitis. Nat Rev Gastroenterol Hepatol. 2020;17(9):533-542.
8. Nelson NP, Easterbrook PJ, McMahon BJ. Epidemiology of Hepatitis B Virus Infection and Impact of Vaccination on Disease. Clin Liver Dis. 2016;20(4):607-628..
9. World Health Organization. "Hepatitis B Fact Sheet". 2022. Archived from the original on 9 August 2022.
10. World Health Organization (WHO). Global hepatitis report. 2017.
11. Block TM, Chang KM, Guo JT. Prospects for the Global Elimination of Hepatitis B. Annu Rev Virol. 2021;8(1):437-458.
12. Chen W, Gluud C. Vaccines for preventing hepatitis B in health-care workers. Cochrane Database Syst Rev. 2005;(4):CD000100.
13. Zuckerman JN. Protective efficacy, immunotherapeutic potential, and safety of hepatitis B vaccines. J Med Virol. 2006;78(2):169-177.
14. Moticka E. A Historical Perspective on Evidence-Based Immunology. 2015;p. 336.
15. World Health Organization. World Health Organization model list of essential medicines: Geneva: World Health Organization. 2019.
16. Narayanankutty A, Palliyil DM, Kuruvilla K, Raghavamenon AC. Virgin coconut oil reverses hepatic steatosis by restoring redox homeostasis and lipid metabolism in male Wistar rats. J Sci Food Agric. 2018;98(5):1757-1764.
17. Chelebi NA, Bazzaz AA, Ali SJ. Potency of natural honey in Homeostasis of Four Liver enzymes in Rats Induced by Doxorubicin. Journal of Food Technology & Nutrition Sciences. 2022;4(2): 1-6.
18. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology. 2007;132(7):2557-2576.
19. El-Serag HB. Hepatocellular carcinoma. N Engl J Med. 2011;365(12):1118-1127.
20. Alter MJ. Epidemiology and prevention of hepatitis B. Semin Liver Dis. 2003;23(1):39-46.
21. Wang H, Men P, Xiao Y, et al. Hepatitis B infection in the general population of China: a systematic review and meta-analysis. BMC Infect Dis. 2019;19(1):811.
22. Premkumar M, Kumar Chawla Y. Chronic Hepatitis B: Challenges and Successes in India. Clin Liver Dis (Hoboken). 2021;18(3):111-116.
23. Australasian Society for HIV, Viral Hepatitis and Sexual Health Medicine. "GESA Australian Consensus Recommendations". Positive- Hepatitis-B for Primary Care. Prevalence and epidemiology of hepatitis B. 2022. Archived from the original on 11 August 2022.
24. Pan American Health Organization. "Hepatitis B and C in the Spotlight. A public health response in the Americas, 2016" (PDF). IRIS PAHO. 2016; Retrieved 11 August 2022.
25. Tello V. High Alanine Aminotransferase (ALT) Causes and health effects. Medically reviewed by Jonathan Ritter, Pharm, PhD (Pharmacology), Puya Yazdi, MD]. 2021.
26. Ishiguro M, Takio K, Suzuki M, Oyama R, Matsuzawa T, Titani K. Complete amino acid sequence of human liver cytosolic alanine aminotransferase (GPT) determined by a combination of conventional and mass spectral methods. Biochemistry. 1991;30(43):10451-10457.
27. Donohue TM Jr. Alcohol-induced steatosis in liver cells. World J Gastroenterol. 2007;13(37):4974-4978.
28. Lala V, Goyal A, Bansal P, Minter D. Liver Function Tests. Stat Pearls. Treasure Island (FL): StatPearls Publishing.
29. Marshall W. Alanine aminotransferase: analyte monograph. Association for Clinical Biochemistry and Laboratory Medicine. 2012; 3-7.
30. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science. 1987;235(4792):1043-1046.