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Enzymatic Alterations in Hepatitis-B Patients within Kerkuk Province, Iraq

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A B S T R A C T

Biochemical alterations in the enzymes of liver from 115 patients admitting Azadi Hospital in Kerkuk province for checkup and treatment were studied and compared with 44 healthy control. The liver enzymes ALT, AST and ALP collected from blood samples were analyzed using ELISA test kit. The frequency of enzymatic abnormality was almost 13%, 22% and 11% for AST, ALT and ALP, respectively. The frequency of hepatitis-B in male patients was higher (59%) than in female (41%). The difference in the first two enzymes were insignificant while ALP revealed significant ($p \leq 0.05$) in comparison with control. However, these differences were significance ($p \leq 0.05$-0.01) when compared between both genders and their control counterparts. The general proportion of infection amongst both genders in Iraq may be similar to those detected in Fareast and Mediterranean countries due to decline in health care in Iraq. The Iraqi females appeared to be more susceptible to infection of hepatitis-B than male counterparts which could be attributed to variation in mode of life.

Introduction

Blood filtration is one of the main function of liver beside metabolizes nutrients, detoxifies harmful substances, makes blood clotting proteins, and performs many other vital functions. It is customary that he most common liver disease, hepatitis-B is characterized as an inflammation of liver tissue via presence of inflammatory cells in the liver where they start leaking out proteins called enzymes that drive these chemical reactions into the blood (Hassan *et al*., 2008; Hunt, 207). These enzymes can then be measured by blood tests by liver function tests (LFT) which include blood check for two main liver enzymes: Aspartate aminotransferase (AST), also found in muscles and many other tissues besides the liver and alanine aminotransferase (ALT) which is almost exclusively found in the liver. LFTs may be performed as a routine blood test to help establish how healthy the liver is or to identify whether a liver disorder may be present and responsible for a person's symptoms. If ALT and AST are found together in an elevated amounts in the blood, liver damage is most likely present (De Ritis *et al*., 2006; Murali *et al*., 2014). The doctor may arrange LFT in certain cases i.e. taking a medication that can harm the liver; liver disease; symptoms of liver or bile system

disease i.e. abdominal pain, nausea and vomiting, or yellow skin, drinking alcohol excessively, exposure to infections such as hepatitis-B. The hepatitis B virus (HBV), is a species of the genus *Orthohepadna virus*, causes the disease hepatitis-B.

Hepatitis, whether symptomatic or none-symptomatic often leads to jaundice, loss appetite, and malaise (Mutuma *et al*., 2011). It could also be either acute or chronic when it persists longer than six months where it may develop to acute liver failure, fibrosis or "cirrhosis". The latter may get further deteriorated to hepato-cellular carcinoma or liver cancer (Carr, 2012). Viral hepatitis, worldwide is considered to be the most common cause of liver inflammation (Chang, 2007) and its complications result in the death of 15- 25% of those with chronic disease (World Health Organization, 2008). The infection can be diagnosed 30-60 days after exposure which could typically be by testing the blood for parts of the virus and for antibodies against the virus (Fischbach *et al*., 2009).

A most recent research indicated that 1/3 of the world population has been infected at one point in their lives, including 240-350 million of chronic infections (Schilsky *et al*., 2013). Over 750,000 people die of hepatitis-B each year and over one third of them are due to liver cancer. The disease is now only common in Eastern Asia and sub-Saharan Africa where between 5-10% of adults have chronic disease. Rates in Europe and North America are, however, less than 1%.[v]The WHO has recorded the world wide total chronic carriers of hepatitis as many as 400 million while the hepatitis-B becomes the most risky amongst other types of hepatitis and does concern all the health organizations worldwide. Recent studies are looking to create foods that contain HBV vaccine as treatment of hepatitis (Thomas, 2013). however, diagnosis deems so necessary by regular periodic check up to insure the health risk. Recently, LFT revealed hormonal changes in blood of patients complaining liver infection within Kerkuk province (Chelebi *et al*., 2016). The objective of this research has been to investigate the changes into liver enzymes ALT, AST and ALP.

Materials and Methods

A total of 115 outpatients, ages ranged 18-70 years old (47 male and 68 female) from both genders admitting regularly to Kirkuk Hospitals for treatment were encountered were involved in this study compared with 44 (24 male and 20 female) healthy volunteers used as control. The patients were further divided into three sub-groups, i.e. 55 patients (29 males and 26 females) with Hepatitis B; 34 patients (9 males and 25 females) characterized by excessive thyroid Hyperthyroidism; and 26 patients (9 males and 17 female) diagnosed by consultants with congestive Hypothyroidism.

Blood samples of 5 mL/patient were collected from all groups via a disposable sterile mediated syringes, and left at room temperature for coagulation. Samples were then centrifuged as 4500 round/min for 10 minutes. Blood serum was saved extracted and saved at -20^{o}C for scanning and serological tests. All serological tests were conducted at the clinical laboratories of General Azadi hospital in Kerkuk.

The surface antigen was screened for hepatitis-B (HBsAg): The ELISA (Plasmatec-USA) test kit was employed according to instructions of the company provided. Blood serum and plasma were analyzed (Noppornpanth *et al*., 2003). Samples were labeled together in wells at

room temperature (18-25^{o}C). The wells of both experimental and 5 controls (3 positive and 2 negative) were labeled. Only 50 mL of conjugated enzymes (HRP+Anti HBs) were added into each blank well. The micro-plate was gently shaken to homogenize the contents, followed by incubation inside an incubator at 37^{o}C for 60 minutes. The wells were then washed 5 times with washing buffer provided within the kit itself. The readily made of 50mL stain-A and 50 mL of stain-B provided by the ELISA company were added to each well. The micro-plate was then incubated at 37^{o}C for 15 minutes. Another 50 mL of reaction stopper of H_2SO_4 was added to each well and gently stirred to complete homogenization. The colour density ocular (OD) off wave length 450 nanometer was read via Micro-Well-Reader. Mean concentration of enzymes ALT, AST and ALP were measured using absorption of negative control and Murine monoclonal standard solutions µlU/ml was measured using rates of optical absorption values for each group via a curved-mediated absorption rate (Soos *et al*., 1982). Biostatistics was performed using Minitab 15 software to analyze mean values and the differences. Data were tabulated accordingly.

Results and Discussion

Generally, the arithmetic values of enzymes AST, ALT, and ALP for hepatitis-B, hyperthyroidism, and hypothyroidism patients in both genders showed significant increases ($p \leq 0.05$-0.01) respectively in comparison with control (Table-1). In only male patients, the mean values of the values of AST, ALT and ALP had slightly but significantly ($p \leq 0.05$) increased in both hepatitis-B and hyperthyroidism but significantly dropped in hypothyroidism in comparison with control (Table-2).

In only female patients, however, the mean values of AST, ALT and ALP enzymes from above three diseases revealed generally a slight difference. The values of both AST and ALT enzymes had insignificantly ($p>0.05$) increased in hepatitis-B, hyperthyroidism but decreased for ALP enzyme. Apart from that other values showed general significant increase (Table-3). The ratio of ALT to AST was almost 1:1 in most cases.

The hepatitis-B incidents vary between country to another pending on the standard of health service e.g. 2% in Western Europe, Northern America and Australia; 2-7% in Eastern Europe, Mediterranean countries, Latin America but had arisen to 8-15% in Far East countries, South African countries. In Iraq, however, it is relatively higher (11-22%) than in Far Eastern countries, South African countries. Such a record denotes serious deterioration of health services and consequence forthcoming health risk. In Iraq, particularly, hepatitis-B increased following three consecutive wars this country was involved in since 1980.

The data presented in this work involved biochemical changes in three different enzymes AST, ALT and ALP in only hepatitis-B, which is most prevalence than other hepatitis C, D, and E in patients attended only to Kerkuk Azadi Hospital as either outpatients or inpatients within a period of 6 months only. For health security, such test deems a must prior any blood donation or reception. Some of these patients has been into the hospitals for general health check up and might have accidentally been diagnosed hepatitis otherwise they could have remained undiagnosed cases. The result showed a relatively higher proportion of infection in Hepatitis-B in male than in female. Similar results were detected in Pakistan, and in

Nigeria, (Ado *et al*., 2010) Turkey (Aydemi *et al*., 2005), India and in Iraq (Ahmad *et al*., 2015) but varied than those in Nigeria (Ali, 2008).

The levels of ALT and AST are two of the most useful measures of liver cell injury, although the AST is less liver specific than is ALT level. The normal range in most clinical laboratories has been found much lower than that for the alkaline phosphatase level which suggests the necessity, when considering levels of elevations, to the respective upper limit of normal for each test compared. Both ALT and AST enzymes exist in certain specialized tissues such as liver, heart and muscles. Any insult i.e. infection to these organs causes release of these enzymes to the blood stream which ends up with increase in their proportion in the blood (Srivastava *et al*., 2007). Such elevation in AST levels are accompanied with other enzymes of heart and liver particularly with ALT (Ali, 2008). The ALP exists in the hepatocytes and involves in the structure of cell membranes and it raises only in liver disorders i.e. blockage of bile duct (Abbas *et al*., 2011). In the case of hepatitis-B, the most detectable increase in the levels of enzymes was in ALT while a moderate increase was noticeable in ALP and AST enzymes. However, in both hyperthyroidism and hypothyroidism the increase in the level of these enzymes was significant in comparison with control although were within the natural limits.

Elevations of the AST level may also be seen in acute injury to cardiac or skeletal muscle. Lesser degrees of ALT level elevation may occasionally be seen in skeletal muscle injury or even after vigorous exercise. Thus in clinical practice, it is not uncommon to see elevations of AST, ALT or both in common non-hepatic conditions such as myocardial infarction and rhabdomyolysis.

Table.1 The mean values of AST, ALT and ALP in micro-gram per liter (u/L) collected from both genders of hepatitis-B, , hyperthyroidism and hypothyroidism patients in comparison with control. (±Sd), Standard deviation; (p), Student T-test biostatistics.

Groups/Parameters	**AST±Sd µ/L**	**ALT±Sd µ/L**	**ALP±Sd µ/L**
Control (n=44)	5.98±0.79	6.16±0.98	50.41±4.81
Hepatitis B (n=55); *p*	6.89±5.15 ≤0.05	7.14±5.75 ≤0.05	61.76±25.44 ≤0.01
Hyperthyroidism (n=34) *p*	6.58±1.41 ≤0.05	6.97±1.67 ≤0.05	58.94±17.80 ≤0.05
Hypothyroidism (n=26); *p*	6.80±1.68 ≤0.05	7.11±2.03 ≤0.05	58.14±15.91 ≤0.05

Table.2 The mean values of AST, ALT, and ALP in u/L for three groups collected from only male patients of hepatitis-B, hyperthyroidism, and hypothyroidism n comparison with control. (±Sd), Standard deviation; (p), Student T-test.

Groups/Parameters	AST+sd µ/L	ALT+sd µ/L	ALP+Sd µ/L
Control A (n=24)	6.00±0.81	6.12±1.02	48.82±4.83
Hepatitis B (n=29); *p*	7.06±5.58 ≤0.05	7.77±5.88 ≤0.05	60.44±26.19 ≤0.05
Hyperthyroidism (n=9) *p*	6.22±1.03 ≤0.05	6.77±1.81 ≤0.05≤	59.38±16.89 ≤0.05
Hypothyroidism (n=9) *p*	5.77±1.54 <0.05	5.66±1.63 ≤0.05	57.71±16.00 ≤0.05

Table.3 The mean values of AST, ALT, and ALP in u/L for three groups collected from only female patients of hepatitis, hyperthyroidism, and hypothyroidism in comparison with control. (±Sd), Standard deviation; (p), Student T-test.

Groups/Parameters	AST+sd µ/L	ALT+sd µ/L	ALP+Sd µ/L
Control (n=20)	5.97±0.75	6.20±0.92	52.32±4.03
Hepatitis-B (n=26); *p*	6.69±4.63 >0.05*	6.45±5.52 >0.05*	63.24±24.49 ≤0.05
Hyperthyroidism (n=25) *p*	6.72±1.51 ≤0.05	7.04±1.61 ≤0.05	58.77±18.11 >0.05*
Hypothyroidism (n=17) *p*	7.35±1.49 ≤0.01	7.88±1.74 ≤0.01	58.37±15.85 >0.05*

Diseases that primarily affect hepatocytes, such as viral hepatitis, will cause disproportionate elevations of the AST and ALT levels compared with the alkaline phosphatase level. The ratio of AST/ALT is of little benefit in sorting out the cause of liver injury except in acute alcoholic hepatitis, in which the ratio is usually greater than 2. These measurements are useful in medical diagnosis to differentiate between causes of liver damage, or hepatotoxicity (Gopal *et al*., 2000; Nyblom *et al*., 2004). In the present research the ratio of ALT to AST has always been 1:1. At this stage it is not clear enough to interpret such a ratio as relevance to any kind of liver dysfunction unless with further tests to the liver referred by medical diagnosis.

The upper limits of serum ALT vary among laboratories however, the upper limit threshold of ALT level should be lowered because people who have slightly raised ALT levels that are within the upper limit of

normal (35-40 IU/L) are at an increased risk of mortality from liver disease (Chernecky *et al*., 2013). In addition, it has been suggested that gender-specific thresholds is applicable because women have slightly lower normal ALT levels than men. In USA the upper limit of ALT was identified of 29 IU/L for men and 22 IU/L for women. This is in concomitant with the present conclusion as gender has been another additional factor of variations in the level of hepatitis patients. In asymptomatic patients with minimal elevations of aminotransferases, it is reasonable to repeat the test in a few weeks to confirm elevation. Elevation scheme in the level of these enzymes denotes multifactoral causes of liver dysfunction the doctors should consider depending upon the profile and history of each patient i.e. race, environment, mode of life, medication, nature of work.

The values of AST detected in hepatitis-B cases are in concomitant with similar work done done in Babylon province in Iraq (Zainal *et al*., 2011) as the levels of both AST and ALT were significantly higher in hepatitis-B than that of ALP which showed a moderate increase. Almost, similar results were detected in hepatitis-B cases at Tikrit province as ALT levels increased significantly higher than AST and ALP. Nevertheless, the present ALT enzyme levels detected in hepatitis-B were in agreement other two studies. However, the changes in the levels of liver enzymes were comparable with most recent study carried out on hepatitis-B in Turkey (Yavuzcan *et al*., 2011). These variations in levels of infection denotes the correlation with the standard of health services and corresponding immune status of the body.

Since 1980s, the infection of hepatitis has become preventable by vaccination (Pungpapong *et al*., 2007). The vaccine works about 95% of the time while an extra 2 or 3 more doses are required at a later time for full coverage. Vaccination is also recommended by the World Health Organization in the first day of life if possible. Accordingly, about 180 countries gave the vaccine as part of national programs as of 2006. It is also recommended that all blood be tested for hepatitis-B before transfusion and usage of condoms guarantees prevent infection. During an initial infection, care is based on the symptoms that a person has. In those who develop chronic disease antiviral medication such as tenofovir or interferon maybe useful, however these drugs are expensive (Stanley *et al*., 2013). Generally, double fold incidence of infection in Hepatitis-B was detectable in Iraq than in thyroidism cases. Such incidence may reflect the fact that hepatitis-B is more predominant than thyroidism.

In conclusion, it is concluded that liver enzymes are susceptible to any subtle infection by hepatitis-B virus which deems so necessary for regular check up for all people as a part of general health security. For an early diagnosis recommendation has been given for clinical double check up for those who may suffer from any kind of liver dysfunction.

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