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Studies on the Liver Function Tests (LFTs) of Obstructive Jaundice, Acute and Chronic Hepatitis Patients

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Abstract: Liver function tests (LFTs) are almost widely used as indicators of hepatobiliary diseases. Activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), asparate aminotransferase (AST) and γ-glutamyl transpeptidase (GGT) as well as total serum bilirubin have been determined in the serum of 68 healthy volunteers (as control) and 184 hospitalized patients suffering from various hepatobiliary diseases. The total serum bilirubin levels were increased significantly (P<0.000) in obstructive jaundice and acute hepatitis patients. In both patient's bilirubin elevation were found about 12 times higher than normal. Where as a moderately significant (P<0.001) elevated level of serum bilirubin up to 4.2 times higher than the normal was found in all chronic hepatitis, patients. Alkaline phosphatase (ALP) was found significantly (P<0.000) high about 4 times greater than the normal serum level, in obstructive jaundice. Both transaminase (ALT and AST) were dramatically and significantly (P<0.000) increased about 48 times higher than in acute hepatitis patients. The ALT/AST ratio was greater than one. In obstructive jaundice and chronic hepatitis patient's transaminase (ALT and AST) were only mildly elevated (P<0.001). γ-glutamyl transpeptidase were significantly (P<0.000) elevated in patients with obstructive jaundice. However, in acute and chronic hepatitis the GGT level were normal or mildly high. All of them were referred for histological study of liver (liver biopsy).

Key words: Liver function tests (LFTs), bilirubin, hepatobiliary, disease.

INTRODUCTION

The composition of blood is almost same in the peoples of different parts of the world. Determination of activity of enzymes in blood for diagnostic purposes was first started from the very beginning of 20th century. By now more than 50 enzymes have been identified in the serum, out of which about 20 enzymes have clinical importance (Wilkinson, 1970). Extensive use of enzyme activity determination as an aid to the diagnosis, monitoring the course and demonstration the sub-clinical evidence of diseases in hospital and clinical laboratories all over the world is one of the most dramatic developments in the modern medicine. Liver is the largest and complex gland of the body. It plays the central role in the metabolism of nutrients. The liver metabolizes endogenous substances such as drugs, which are then excreted via the kidney or biliary system (Guyton, 1991). It is also the biggest reticuloendothelial system of the body and as such has immune functions in maintaining body integrity (Sherlock and Dooley, 1993). Signs and symptoms of liver diseases often do not become apparent before a significant portion of the liver is damaged. For this reason, practitioners are trying to find ways and means for the proper diagnosis of liver diseases. Biochemical tests, commonly known as liver function tests (LFTs) are widely used for diagnostic purposes, which provide simple, easily assessable noninvasive means of detecting and monitoring abnormalities of liver function and cellular integrity (Moss, 1900). That usually increases during liver damage by surgery or injury or disease condition. Symptomatic acute liver disease, which is often viral, produces generalized symptoms of malaise, anorexia and fever. Jaundice may appear as the disease progresses. Jaundice is a yellow coloration of the skin and mucous membranes (Kumar and Clark, 1994). It is detectable when the serum bilirubin is greater than 3 mg dl^{-1}. Hepatitis is a term used to refer the inflammation of the liver (Popper, 1986). Viral Hepatitis caused by virus, classified serologically in to five categories. Hepatitis A Virus is the commonest type of viral hepatitis and causes 20-40% of clinically apparent hepatitis. This virus causes acute hepatitis usually in children and young adults (Dame and John, 1991). It is usually spread by fecal oral route and also can be follow transfusion of blood from donor who is in the incubation stage of the disease (Hollinger *et al.*, 1983). Type A hepatitis occurs throughout the world with the highest incidence in

countries with low standards of sanitation (Ajimi *et al.*, 1986).

Viral hepatitis is the commonest alarming liver disease occurring sporadically rounds the year alongside the common clinical burden of cirrhosis of liver and hepatocellular carcinoma (Rector, 1992). Around six to seven percent of the total populations of our country are carrying hepatitis B virus. Three out of 10 hepatitis B patients suffering from liver damage which gradually push the patients towards death if the patients were not treated properly (Anonymous, 2000). So HBV is the main etiological factor for chronic liver disease in Bangladesh. In the northern region of our country, the frequency of the viral hepatitis would be more pronounced according to the Rajshahi Medical College Hospital sources as well as expert physicians in this region. Acute hepatitis is very common and death occurs resulting from chronic hepatitis. The incidence of cirrhosis and hepatocellular carcinoma is in a rising state in our country. However, accurate statistical data was not available.

We intended to study on the liver function tests include measurement of serum activity of different enzymes mainly the transaminase i.e alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT), as well as bilirubin level in the serum of hepatobiliary patients, in comparison with individuals having no history of hepatitis or jaundice. The main object of our present study was to find out the relationship among ALP, ALT, AST, GGT and bilirubin level in the serum of patients with obstructive jaundice, acute hepatitis as well as chronic hepatitis with healthy individual as controls.

MATERIALS AND METHODS

Materias: All chemicals and reagent kits for this study were collected from British Drug House, U.K; Merck, Germany; Bio-Rad Laboratories, Richmond, U.S.A; Sigma Chemicals Co., U.S.A; Pointe Scientific Inc., Michigan, U.S.A; and Randox Laboratories Ltd., Antrim, U.K.

Sixty-eight (68) healthy human subjects-24 men (adult), 22 women (adult) and 22 children were chosen as control. Of the control humans, 16 non-smokers and eight were smoker. All 22 women were non-smoker, non-pregnant and 22 children were non-smoker. From these volunteers, blood samples were collected for the assays of ALP, ALT, AST, GGT and total bilirubin. Blood samples were randomly collected from 184 hospitalized patients from Rajshahi Medical Collage Hospital (RMCH) and three City diagnostic centers, who were suffering from various liver diseases. Among them 89 were obstructive jaundice patients, 56 were acute hepatitis patients and 39 were chronic hepatitis patients. Both the healthy (control) and patients were grouped into four groups.

For the determination of the activity of serum enzymes blood samples from control and using sterile disposable syringe with 21 gauge needles drew patients. Samples from control and patients about 5 ml each, were taken into clean and dry centrifuge tubes (1x1 cm), placed in to ice box. The blood samples taken into centrifuge tube, allowed to clot reaction and then centrifuged for 10-15 min at 4000 rpm. The serum samples storage in deep freeze for further assayed experiments.

Methods: Assay of Alkaline Phosphatase (ALP): The ALP activity was determined by the method of Bessey *et al.*, 1946). A slight modification of the procedure was made. The buffer 2-amino-2-methyl-1-propanol was replaced by bicarbonate buffer and instead of recording 2 min of linear reaction by the automated spectrometer, the activity was determined from the increase in absorbance at 405 nm due to the formation of the reaction product p-nitrophenol and also by a commercially available reagent kit methods (Fujita, 1969; Bessey *et al.*, 1946; Bowers *et al*., 1966; Anonymous, 1974).

Assay of alanine aminotransferase (ALT): This enzyme also known as Glutamic-Pyruvic Transaminase (GPT). Serum alanine-aminotransferase (ALT) was determined by the Recommended method of the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Physiology (Anonymous, 1974) and by a commercially available reagent kit (Reitman and Frankel, 1957), respectively.

Assay of aspartate aminotransferase (AST): The enzyme also known as Glutamic-Oxaloacetatic Transaminase (GOT). Serum AST was determined by the Recommended method of the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Physiology (Anonymous, 1974) and by a commercially available reagent kit, respectively (Reitman and Frankel, 1957).

Assay of serum γ-glutamyl transferase (γ-GT/GGT) activity: GGT was assayed by the method of (Rosalki and Tarlow, 1974) and by a commercially available reagent kit (IFCC, 1983), respectively. Determination of serum Bilirubin by a commercially available reagent kit (Jendrassik and Grof, 1938; Poper and Schaffiner, 1995).

RESULTS AND DISCUSSION

Activity of alkaline phosphatase (ALP) alanine aminotransferase (ALT), aspartate aminotransferase

(AST), γ-glutamyl transpeptidase (GGT) and bilirubin level have been determined in random serum samples from 68 controls and 184 patients with three disorders of liver and biliary system. Mean serum ALP, ALT, AST and GGT activities as well as bilirubin level determined among the healthy person were shown in Table 1.

The level of serum ALP activity in control children group that we found were higher than that in males and females are in good agreement with other workers (Fleisher *et al.,* 1977; Reyes *et al.,* 1978). The highest activity of serum ALP in children is due to bone formation and that of the males than females are due to higher muscular activities. The serum level of ALT activities were found within the range between 4-12 U L^{-1} among the control group which shown in Table 1. The activity of ALT in female and children were found closely similar but the value was slightly higher (3%) in the male control group however the difference is not significant. The slightly difference in ALT activity in between male and female, being slightly higher in male was also shown by Wilkinson *et al.* (1972).

The normal serum AST level that we observed also varying from 4-12 UL^{-1} in all three-control groups. The normal serum AST activity among the children was found slightly higher than that in male and females; the AST activity in the male and female were closely similar. Bohnen *et al.* (1992), reported that a slightly elevated level of serum AST were found in children than in male and female and no difference in sex.

By observing the results that we obtained that the serum levels of GGT activity among the healthy males are higher than (-54%) that in females and children (Table 1). This finding correlated with the study of IFCC expert panel on Enzyme (1983).

The lower level of total serum bilirubin among the control groups was found 0.28 mg dl^{-1} and that of the upper level was 1.2 mg dl^{-1}. These determined normal serum levels showed a good agreement with the Jendrassik and Grof (1938). 0recommended normal serum level of total bilirubin unto 1.0 mg dl^{-1}. Moreover we found slight variation in the bilirubin level with respect to sex differences and age being slightly higher in male than that in female and children. Fevery *et al.,* (1986) found sex differences in the level of serum bilirubin with reference limit varying from 0.8-0.95 mg dl^{-1} in females and 1,18-1,4 mg dl^{-1} in males.

The serum levels of ALP were significantly ($P< 0.000$) higher in three categories of patients as compared to control groups. These obtained result are in good agreement with the findings of (Poper and Schaffiner, 1995; Ricketts *et al.,* 1952; Shay *et al.,* 1945 and Welin, 1941) who observed that serum ALP was elevated up to 2 to 3 times higher than the normal in obstructive jaundice patients.

Table 1: Estimated value of different biochemical parameter in control (healthy) persons

Biochemical marker	Male (adult) (Mean±SE) n=24	Female (adult) (Mean±SE) n=22	Children (Mean±SE) n=22
ALP (UL^{-1})	195.5±10.89	172.5±9.29	476.40±25.20
ALT (UL^{-1})	8.0±0.38	7.77±0.36	7.70±0.38
AST (UL^{-1})	8.04±0.34	7.81±0.40	8.18±0.40
GGT (UL^{-1})	34.08±1.80	23.13±1.23	22.60±1.17
Bilirubin mg dl^{-1}	0.68±0.03	0.63±0.04	0.46±0.04

Table 2: Estimated value of different biochemical parameter in obstructive Jaundice

Biochemical marker	Male (adult) (Mean±SE) n=36	Female (adult) (Mean±SE) n=31	Children (Mean±SE) n=22
ALP (U L^{-1})	591.97±21.73	526.19±27.59	1342.27±53.81
ALT (U L^{-1})	48.94±3.49	46.90±3.89	46.04±4.28
AST (U L^{-1})	49.66±3.50	48.80±4.26	49.75±5.59
GGT (U L^{-1})	453.58±45.19	308.96±31.29	311.90±36.91
Bilirubin mg dl^{-1}	7.73±0.69	6.92±0.70	7.15±0.94

Table 3: Estimated value of different biochemical parameter in Acute Hepatitis Patients

Biochemical marker	Male (adult) (Mean±SE) n=25	Female (adult) (Mean±SE) n=19	Children (Mean±SE) n=12
ALP (UL^{-1})	386.32±29.07	343.68±24.67	712.0±76.43
ALT (UL^{-1})	384.8±54.10	378.52±59.2	374.5±72.94
AST (UL^{-1})	347.4±48.96	333.16±57.89	364.16±68.44
GGT (UL^{-1})	170.6±12.86	115.26±9.91	113.75±8.64
Bilirubin mg dl^{-1}	8.01±1.06	7.62±1.27	8.04±1.55

Table 4: Estimated value of different biochemical parameter in Chronic Hepatitis Patients

Biochemical marker	Male (adult) (Mean±SE) n=20	Female (adult) (Mean±SE) n=14	Children (Mean±SE) n=5
ALP (U L^{-1})	234.4±15.25	206.07±13.37	605.0±36.92
ALT (U L^{-1})	53.1±5.69	48.5±6.55	52.8±11.22
AST (U L^{-1})	47.9±5.22	46.35±6.41	50.2±9.33
GGT (U L^{-1})	59.6±11.98	37.35±5.01	37.2±5.72
Bilirubin mg dl^{-1}	2.83±0.19	2.63±0.22	2.93±0.44

The mild to moderately elevated levels of both serum transaminase (ALT and AST) as shown in Table 2 were within the range from 2-9 times higher than the corresponding upper limit of normal among all the obstructive jaundice patients. It was observed that the elevated ALS activities were found within the range from 22-94 UL^{-1} and that of the AST were between 24-98 UL^{-1} among all the obstructive jaundice patients.

Significant elevation ($P<0.000$) was found in the serum GGT levels of the patients with obstructive jaundice. The fluctuating elevated level of serum GGT that we determined ranging from 135-1025 UL^{-1} among the patients with obstructive jaundice. It was also observed that the elevated GGT levels in all three categories of obstructive jaundice patients were in between 3-22 times higher than the normal serum level (Table 2). The similar observations were also reported by Goldberg (1991) who found the highest activity of serum GGT in intra-or post

hepatic biliary obstruction (obstructive jaundices) with increase being generally greater in intrahepatic cholestasis than in extra hepatic cholestasis.

Results given in Table 2 shows a significant (P<0.000) rise in serum bilirubin level in all three categories of obstructive jaundice patients. In all patients with obstructive jaundice, the fluctuating level of serum total bilirubin as determined range from 2.1-18.05 mg dl^{-1}. In all the male hepatitis patients, the elevated serum level of ALP activity was moderately significant (P<0.009). The elevated ALP activities were observed within the range from 180-1120 UL^{-1} in patients with acute hepatitis. Among the acute hepatitis patients the elevated serum level of ALP activity were observed below 2.4 times higher than the upper limit of normal serum level (Table 3).

In all the patients with acute hepatitis, both the transaminases i.e ALT and AST were found significantly (P<0.000) and dramatically elevated. The ALT elevations were reported in, between 110-1045 UL^{-1} among the acute hepatitis patients, respectively. While the AST elevation were slightly lower than that of ALT, ranging from 97-940 UL^{-1} among the male, female and children acute hepatitis patients. The ALT and AST elevation were found 8-80 times higher than the upper limit of normal among the all acute hepatitis patients.

In acute hepatitis, the serum GGT levels were found mild to moderately elevated. The elevated levels were up to about 5 times higher than the upper limit of normal in all categories of obstructive jaundice patients. The elevated GGT levels were ranged from 65-320 U L^{-1} among the male, females and children acute hepatitis patients. Spectacular and significant (P<0.000) elevation in serum total bilirubin was observed among the patients with acute hepatitis. The elevated bilirubin level ranging from 2,25-19.75 mg dl^{-1} among the patients with acute hepatitis. The elevated levels were found up to 15 times higher than the upper limit of normal in all acute hepatitis patients.

In patients with chronic hepatitis only mild to moderate elevation in serum ALP levels were found and also the elevation was less significant (P<0.01). The elevated ALP level were found in between 155-965 UL^{-1} among the males, females and children patients with chronic hepatitis. The elevated ALP levels were observed below two times higher than the upper limit of normal. Serum transaminase elevation was also found significant (P<0.001) among all the chronic hepatitis patients.

Among all the chronic hepatitis patients (males, females and children) the elevated transaminase levels were found within the range below 8.2 times higher than the corresponding upper limit of normal serum level.

A mild to moderate elevation in serum GGT level were notable in chronic hepatitis patient group, the elevation is moderately significant (p<0.009). In chronic hepatitis patients, the estimated value of serum GGT were found varying from 25-88 U L^{-1}. The elevated GGT levels were found less than two times higher than the upper limit of normal among the chronic hepatitis patients (Table 4).

Although in chronic hepatitis patients the elevation in serum total bilirubin is significant (P<0.001), but a fluctuating elevated values were recorded in our chronic hepatitis patients group and most values were in below 5.00 mg dl^{-1} (up to 4 times higher than the upper normal limit). Among the chronic hepatitis patients the serum total bilirubin were found within a broad range between 1.6-4.55 mg dl^{-1} in male, female and children chronic hepatitis patients.

In liver diseases of any sort, serum bilirubin level is elevated. In general, serum bilirubin reflects the depth of jaundice and repeated estimation may be useful in following the prognosis of the diseases. Alkaline phosphatase (ALP) and γ-glutamyl transpeptidase (GGT) increase because of obstruction in the bile flow due to metastases or any other cause, while transaminase increase as a result of hepatic cell necrosis. Among the obstructive jaundice patients the total serum bilirubin, alkaline phosphatase (ALP) and γ-glutamyl transpeptidase (GGT) markedly increase, while the transaminase mild to moderately increase. In acute hepatitis, bilirubin and transaminase increase markedly but ALP and GGT activity normal/only mildly increases. In chronic hepatitis, transaminase and bilirubin increases moderately. ALP and GGT may be normal/mildly elevated. Use of serum enzyme or other biochemical tests for diagnostic purposes depends on a number of factors. Age, sex, exercise and pregnancy very often influence enzyme levels. The half-life of an enzyme is very important, variation in the rate of clearance has an important contribution on the use of biochemical tests in diagnosis. To increase the reliability of enzyme tests for diagnostic purposes in our country, intensive work should be carried out (undertake) in this field. If large numbers of patients (samples) are studied for a particular disease, the result will be more accurate.

This investigation therefore, can be regarded as a small population. Besides this four enzymes and bilirubin, may other enzymes and constituents in the blood, which deserve extensive investigation for their proper application in clinical diagnosis.

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