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RESEARCH ARTICLE

DIAGNOSIS OF THE LIVER ENZYMES USED FOR THE CONFIRMATION OF A PARTICULAR TYPE OF JAUNDICE

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Abstract

More than 25 million people in the United States suffer from liver and gallbladder diseases, according to the American liver Foundation. And, more they 43,000 die of a liver disease each year. The bilary system-consisting of the bile ducts and the gall-bladder, and the pancreas are all also closely associated with the functioning of the liver. Some liver, biliary, and Pancreatic diseases are congenital. Others can be prevented. In any case, whether these diseases are congenital injury, viral-induced, or alcohol- induced, they can be devastating to a person's health and require professional care.

Key words: liver and gallbladder diseases, American liver Foundation, Pancreatic diseases

Introduction

Liver dysfunction has been found in 81 % of postpartum women in the general population. This disjunction was speculated of sub clinical autoimmune hepatitis. Therefore 100 methods were developed for the detection of auto antibodies to liver-specific antigens. An Elisa for anti-liver-specific organize antibodies and a highly sensitive radioligand assay for anti-cyp2D6 anti bodies. Basic examinations of dilution curve, inhibition study and reproducibility were satisfactory for clinical application in both assays. Anti – organsae antibodies and anti-cyP2D6 antibodies were found autoimmune hepatitis, respectively. There was no correlation between the two autoantibodies and thus, combined use of these antibodies detects 55.3% of autoimmune hepatitis. Autoimmune hepatitis exists frequently when we include mild cases.

In 1997, liver disease was the tenth most common cause of death in US; more than 25,000 people died that year because of liver disorders.

Some common liver disease symptoms include

Jaundice
Cholestasis
Liver enlargement
Portal hypertension
Ascites
Liver encephalopathy
Liver failure

Liver enlargement (hepatomegaly) is usually an indicate of liver disease. There are usually no symptoms associated with a slightly enlarged liver.

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Liver failure

Liver failure is severe deterioration of liver function. Liver failure occurs when a large portion of the liver is damaged due to any type any liver disorder. Symptoms may include.

Jaundice
Weakness
Nausea
Loss of appetite

Jaundice comes form the French work Jaune, which means yellow. Jaundice is the yellowish discolouration of the eyes (sclera) & skin (advanced cases). Scientifically speaking it is the condition associated with abnormally high levels of bilirubin.

Bilirubin is the breakdown product of Hemoglobin. Hemoglobin is the oxygen carrying component present in the red blood cells. When re blood cells are worn out, they are broken down & bilirubin is formed. Liver plays an important role in this process. The bilirubin is then excreted in the bile. Bile is a fluid that is stored in the gallbladder, then discharged through biliary ducts into the small intestine, where it aids in the digestion of facts.

Normally the bilirubin is removed from the bloodstream by the liver and eliminated from the body in the bile, which passes from the liver into the intestines. If the amount of bilirubin build up in the blood, the skin and whites of the eyes which contain numerous small blood vessels they become yellowish. There are several conditions that may interrupt the elimination of bilirubin from the blood and cause jaundice. Small or moderate increase in billirubin are not harmful. Bilirubin levels increase over the first several days and then fall slowly Extremely high levels of bilirubin can be harmful, causing brain damage.

Jaundice

Jaundice is a yellow discoloration of the skin and eye whitens due to abnormally high levels of bilirubin in the blood stream urine is usually dark because of the bilirubin excreted through the kidneys high levels of bilirubin may be attributed to inflammation on other abnormalities of the liver cells or blockage of the bile ducts. Sometimes jaundice is caused by the breakdown of a large number of red blood cells. Which is common in newborns but can occur in adults. Jaundice is usually the first step and sometimes the only sign, of liver disease.

Types of jaundice are

Hemolytic jaundice (Pre Hepatic jaundice)
Obstructive jaundice (Post Hepatic jaundice)
Hepatocellular jaundice (Hepatic jaundice)

Hemolytic jaundice

Is a type of jaundice over when there is excessive destruction of red blood cells. In that case, due to destruction too much of bilirubi9n is formed, and other of anemia and in some infectious diseases like malaria, sometimes come from an immune reaction of a baby to antibodies in its mother's blood, producing severe jaundice at birth. Bilirubin is the major product of heme from hemoglobin, about 70% of which is derived from senescent red cells (Crawford and *et al*, 1988).

Obstructive jaundice

After being processed by the liver, bilirubin is excreted in the bile. If there is any obstruction to the flow of bile, the bilirubin would be pent up in the body; usually stone or stricture of the bile duct blocks the passage of bile from the liver into the intestines. Sometimes newborns have

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jaundice as a result of a congenital obstruction of the bile ducts.

Hepatic jaundice

This is a jaundice in which the problem lies with the liver. Due to infection of the liver, the bilirubin is not processed. Liver cells are damaged by diseases such as hepatitis or cirrhosis of the liver; the damaged liver is unable to remove biliubin from the blood.; thus allowing bilirubin levels in the blood to build up Alcoholism and prolonged alcohol abuse often lead to a breakdown in liver function, resulting in jaundice. A bout 15% derives from hepatic sources and a minor amount derive from kidney and bone marrow.

Bilirubin production in healthy adults averages 250 to 350mg / day (Muraca and *et al*., 1982). Various amounts of serum bilirubin circulates in plasma bound to albumin so clled delta_bilirubin (Lauff *et al*., 1982). Which becomes elevated particularly in patients with chlestatic jaundice (Vanhootengem, 1985).

The diagnosis of the liver enzymes are used for the confirmation of a particular type of jaundice. For liver function tests assessment of three enzymes are determined. Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate tranaminase (SGPT) is a mitochondrial enzyme relased from heart, kidney etc. SGPT is a cytosolic enzyme primarily present in the liver, transaminase estimation are useful in the early diagnosis of viral hepatitis. Similarly ALP is present in several tissues including liver, bone, kidney, intestine and placenta. ALP in the liver exists predominantly in the biliary tract and is therefore a market for biliary dysfunction.

Aim of study

The study aims at evaluating biochemical parameter Pertaining to Jaundice of varied etiology.

The parameters chosen for study are as follows.

Total bilrubin
Direct bilnubin
SGOT
SAPT
Alkaline phosphates
Total protein
Albumin
Globulin
A/G ratio

Material and methods

Study group:-

Serum sample of about 30 patients suspected to jaundice, clinically had collected.

Control group:-

For control group about 10 serum samples had collected from normal healthy individuals.

Sample Collection

Serum samples from about 30 patients suspected to have liver disorder had collected from medical trust Hospital Cochin

From each patient 5 ml of venous blood it was collected in sterile disposable syringe and it was allowed to clot. After centrifugation serum is collected from the clotted blood. That serum used for the analysis by the following biochemical parameters.

Total bilrubin
Direct bilnubin
SGOT
SGPT
Alkaline Phosphatase
Total protein
Albumin
Globulin
A/G ratio

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Determination of Direct and In direct Bilirubin
Serum bilirubin was estimated by the methods of Malloy and Evelyn (1937)

Procedure

Two test tubes were taken and into each was placed 0.2ml of serum and 1.8 ml of distilled water. To the unknown added 0.5 ml of Diazoreagent and to the blank 0.5 ml of 1.5 percent Hydrochloric acid Finally to each added 2.5 ml of methanol. Allowed to stand for thirty minutes and read in the colorimeter using a yellow-greenfilter. Subtracted the reading of the blank from that of the test.

The amount of direct reacting bilrubin was determined in a similar way by substituting 2.5 ml of water for the 2.5 ml of methanol.

Procedure

Washed 0.2 ml of serum into 5.4 ml of water and mix. For values above 15 mg per 100 mλ. 0.1 m of serum and 5.5 ml of water can be used. Pipette 2.8 ml of this into a second tube for use as the blank. To the test add 0.7 ml of diazo-reagent and to the blank 0.7 ml of sulphanilic acid solution mixed and allowed to stand for five minutes and read at 540 Mill microns or using green filter. This gives the conjugated bilinubin. Then to obtain total bilrubin added 3.5 ml of methanol to each tube and read again after standing five minutes. As the standard added 0.2 ml of bilrubin standard to 3.5 ml methanol. Then added0.7 ml of diazo-reagent and after mixing 2.6 ml of water and read against a water blank for five minutes.

Results

Results were expressed as mg / dl Mg. Conjugated bilrubin per 100 ml

$$\frac{\text{Reading of unknown}}{\text{Reagent of standard}} \times 10 \times 1.05$$

Conjugated bilirubin has a lower extinction in water so the factor 1.05 has to be in serted

$$\text{Total bilirubin} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 20$$

The difference gave indirect bilirubin

Determination of serum glutamate oxaloacetate transaminase activity (SGOT)

Serum SGOT was estimated by spectrophotometric method by Karmen (1955)

Procedure

For the test mixed 0.2 ml of serum , 1.7 ml as 0.5 ml of asparate, 0.3 ml of $NADH_2$ and 0.1ml of malate dehydrogenase in a cuvette with a light path of 1 cm, and for the blank 0.2ml serum, 2 ml buffer, 0.5 ml asparate and 0.1 ml of malate dehydrogenase. Allowed to stand for is to 15 minutes that is until the fall in reading due to partial oxidation of $NADH_2$ layers off. Then added 0.2 ml of alpha-oxaglatara to the test , mixed well and read against the blank at minute interval for 10 minutes. At the end of this time take the temperature at the test mixture. Some increase in extinction occurs due to absorption of light by the oxoglutarate at this wave length after its addition. This is followed by a steady decrease.

The unit of activity is that which produces a decrease in extinction of 0.001 and is expressed per ml. Of serum per minute. Hence in the above method.

Units per ml per minute = decrease in extinction in 5 minutes × 1,000

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Results

The activity of serum glutamate oxaloacetate transaminase was expressed in μg/min/ml of serum.

Determination of serum glutamate Pyruvate transaminase activity :

Serum SGPT was estimated spectro photometrically by Henry *et al*., (1960).

Procedure

Varying μl of standard was pipetted out into a series of test tube. Then it was made upto 1 ml with buffer 1ml of the buffered substrate was pipetted out into the control 0.2ml of serum was added to the test solution. Thus these were incubated at 37°C for one hour. 2 drops of aniline citrate reagent was added after incubation. 0.2ml of serum was added to the control to all the tubes 1ml of dinitrophenyl hydrazine was added and 10 ml of 0.4 N NaoH was added. After 20 minutes against developed was read at 520 nm against reagent blank.

Result

The activity of SGPT was expressed in μg/min/ml of serum.

Determination of serum Alkaline phosphates

Serum alkaline phosphatase was estimated by king and Armstrong (1934)

Procedure

Pipetted 6 ml of the buffer substrate into a test tube and placed in the water bath at 37°C for a few minutes. Added 0.3 ml of serum Preferably without removing form the bath. Mix and cork and allowed to remain in the bath exactly fifteen minutes. Removed and immediately added 2.7 ml of the diluted phenol reagent. At the same time set up a tube for the control containing 6 ml of substrate and 0.3 ml of serum, to which is added immediately 2.7 ml of diluted phenol reagent. Placed the three tubes in the 37°C water bath for fifteen minutes and read on the colorimeter. As blank taken 2.8 ml of water and added 1.2 ml of diluted phenol regent 1 ml of 20 percent sodium carbonate. A red filter is used with transmission at 680 milli microns.

Result:

The result was expressed in mg of phenol liberated by 100 ml of serum of fifteen minutes at 37°C

Determination of proteins in serum:-

Serum proteins was estimated by Kingsely (1942) using biuret method

Procedure

carried out all stages up to the point at which the colour is developed with the biuret reagent, at a temperature above 25°C otherwise sodium sulphate may crystallize out. This can be conveniently by precipitating the globulins in a centrifuge tube. Placed inside a wider centrifugal tube containing water warmed to about 30°C and centrifuged both tubes.

Total proteins

pipetted 6.0 ml of the sulphate sulphite solution into a 90X15 mm. Centrifuged tube and on to it layer 0.4 ml of serum. Inverted and mixed then removed at once 2 ml of the mixture and it to 5 ml of biuruet mixture in a test tube.

Albumin

Added about 3 of ether to the rest of the serum sulphate- sulphite mixture, stopped shake. Shake forty times, twice each second for twenty seconds, using a movement of the arm of about 15 inches. It

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is important not to shake more vigorously otherwise the albumin way be denatured. The time of shaking should not exceed twenty five seconds or less than fifteen seconds. It is important not a shake mere vigorously otherwise the albumin may be denatured. The time of shaking.

Should not exceed twenty – five seconds or be less than fifteen seconds centrifuged for five minutes, thatn is, just long enough for a form globulin layer to form. Caped the tubes during the centrifuging. Rubbercaps were convenient. The air space in the tubes should be less than 3 ml. If it is larger some of the albumin may be denatured and two results will be obtained. After centrifuging till the tube and insert a pipette into the clear solution below the globulin layer . taken care not to disturb the precipitate. Pipetted 2 ml of this and added to 5 ml of the buret reagent in a test tube.

In addition set up the following tubes.

serum blank

Added 2 ml of serum- sulphate-sulphite mixture to 5 ml. Of the tart trate - iodide solution and mixed. This should be put up whenever the serum is opalescent or abnormally pigmented.

Biuret blank

Added 2 ml of sulphate-sulphate solution to 5 ml of the biuretle reagent.

Standard

pipetted 0.4 ml of the standard serum into 6.0 ml of sulphate – sulphite solution as above and transferred 2 ml of the mixture in to 5 ml of the biuret reagent in a test tube.

Standard serum blank:

Prepared this as described for the test serum. Shaken then placed the tubes in a water bath at 37°C for ten minutes. Allowed to cool for five minutes at room temperate then read in the absorption at 555 Milli- microns or using a Yellow-green filter- Read the serum blanks against the tartar ate iodide solution and the test and standard against the biuret blank.

Total protein and albumin were obtained the difference between these gives total globulins

Results

The protein in sample was expressed in grams and the albumin globulin ratio was expressed in percentage.

Results and statistics

Total bilirubin, Direct bilirubin, SGOT, SGPP, alkaline phosphates, total protein, albumin, globulins, A/G ratio in different liver disorders like hemolytic abstractive and hepatic jaundice has been done collecting 50 samples from medical Trust Hospital cochin

Out of 50 subjects 29 subjects were classified into 3 groups. 9 subjects were suspected to have hemolytic jaundice. 10 subjects were suspected to have hepatic jaundice and 10 obstructive jaundice. So they were grouped as study group.

The normal level of bilirubin (Total) is 0.00 – 1.0 mg / dl and of direct bilirubin is 0.0 – 0.3 mg / dl. The first column shown bilirubin level of normal patients.

Elevations can be seen in 3 types of Jaundice. In HE MOLYTIC JAUNDICE the bilirubin levels are high due to increase in the break down of RBC'S

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Table. I Comparative elevated rations of direct and indirect bilirubin in different types of Jaundice

Class	Direct bilirubin Mean ï S.D	Total bilirubin Mean ï S.D
Normal Hemolytic Hepatic Obstructive	0.164 ± 0.127 7.82 ± 5.49 4.4 ± 2.46 8.10 ± 5.1	0.65 ± 0.33 8.15 ± 6.81 3.94 ± 3.03 8.80 ± 6.5

Table II shows the changes in the variations of transaminases in the jaundice patients.

Class	SGOT	SGPT
Normal Hemdytic Hepatic Obstructive	26.7 ± 5.57 32.11 ± 8.0 199.6 ± 190.53 32.5 ± 4.19‘	46.7 ± 12.43 52.11 ± 4.19 187.9 ± 154.58 53.5 ± 8.14

Table III shows a high level of alkaline phosphates in obstructive jaundiced patients. It is found to be 5 fold than that of normals.

Class	SGOT
Normal Hemdytic Hepatic Obstructive	68.8 ± 19.18 119.11 ± 10.06 99.7 ± 18.12 597.6 ± 1248.9

Table IV shows that proteins are into a very sensitive indicators of liver diseases.

Class	P.P	G / b	A / b	A / G
Normal	7.1 ± 8.3	3.41 ± 0.58	3.68 ± 0.52	1.22 ± 0.19
Hemolytic	7.03 ± 1.24	3.3 ± 0.8	3.56 ± 0.7	± 0.32
Hepatic	5.41 ± 2.06	3.36 ± 0.67	3.59 ± 1.68	1.07 ± 0.26
Obstruction	7.1 ± 0.83	3.41 ± 0.58	3.68 ± 0.52	1.22 ± 0.191

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In pre-hepatic jaundice it is the in ability of the liver to handle an increased bilirubin load.

The second column shows increase in hepatic Jaundice. It includes acute hepatitis, hepatotoxity and alcoholic liver diseases.

In obstructive jaundice since there is obstruction in the flow of bile to the intestine there is rise in both direct and total bilirubin.

Transaminases

Table II shows the changes in the variations of transaminases in the jaundice patients. The first column indicates the mean and the standard deviation of the normal patients.

The second column shows a slight increase than that of normal. The mean and standard deviation found to be 32.11 ± 8.0 and 52.11 ± 14.19 yet they are within the normal range.

But in hepatic jaundice the increase was found to be fold than that of the normals. It indicates acute hepatocellular disease.

The third column shows a minimal change in the level of enzymes. The mean and the standard deviation was found to be 32.5 ± 4.19 and 53.5 ± 8.14 respectively. There is only minimal change from that of the normal. Extra hepatic obstruction usually does not cause high rise in the level of enzymes. The largest elevation in excess 3 observed in acute viral hepatitis.
Table III shows a high level of alkaline phosphates in obstructive jaundiced patients. It is found to be 5 fold than that of normals. The mean and the standard deviation in obstructive jaundiced patients was found to be 597.6 ± 1248.9 respectively.

In intra and extra hepatic biliary obstruction alkaline phosphates is elevated before jaundice develops. The obstruction may be due to stoner, tumors fibrous, granulomas.

There is no level of increase in hemolytic and hepatic jaundiced patients. The mean and standard deviation was found to be 119.11 ± and 99.7 ± 18.12 respectively. Alkaline phosphatese usually does not tise in hemolytic and hepatic patients.

Table IV shows that proteins are into a very sensitive indicators of liver diseases. It has limited values for differential diagnosis and abnormal values may be also seen in other Hon hepatic disorder.

The rate of hepatic albumin synthesis falls in the face of inadequate proteins intake. This is a frequent occurrence in patients with advanced liver diseases and particularly those in whose excessive alcohol consumption is implicated.

Even when the rate of synthesis falls, plasma levels may remain within the reference range because of a compensatory reduction in the rate of degradation. Furthermore, hypoalbuminaemia may occur in the face of normal or even increased rates of synthesis when protein leaks into lymph, sautés or otherwise into the extra vascular commandment.

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