Research Article



Diabetes Mellitus as a Risk Factor in Patient with Liver Cirrhosis and Hepatocellular Carcinoma - Biochemical Studies

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ABSTRACT

AS hepatocellular carcinoma (HCC) is a progressive disease, in this study we report the effect of diabetes mellitus (DM) on patients with liver cirrhosis (LC) and hepatocellular carcinoma (HCC) via monitoring various of biochemical markers such as blood sugar, Alpha-fetoprotein (AFP) and liver function (alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, total bilirubin (TB) and direct bilirubin (DB). The experimental involved three groups of voluntary Egyptian aged between 18-60 of males and females. Group 1: Healthy control: served as healthy subjects and they did not show any clinical or biochemical disorders. Group 2: patients with liver cirrhosis associated with diabetes mellitus (DM) and group 3: patient with hepatocellular carcinoma associated with diabetes mellitus (DM). Results showed that glucose intolerance was the main reason behind increasing the level of random blood sugar in second group. It was also observed that diabetes mellitus is associated with about two to three fold increased risk of HCC and increasing the risk of death from HCC in the large cohort study.

Keywords: Liver cirrhosis (LC); Diabetes mellitus (DM); Hepatocellular carcinoma (HCC); biochemical markers.

INTRODUCTION

irrhosis is a result of disease of the liver. It is marked by replacement of liver tissue with scar tissues and fibrosis, leading to a loss of function in the liver due to long-term damage. Typically, the disease develops slowly over months or years with often no symptoms according to Lancet 2015¹. The signs and symptoms may be either a direct result of the long term failure of liver cells, or secondary due to the resultant portal hypertension. As the disease worsens, tired, weak, itchy, have swelling in the lower legs, yellow skin, fluid build-up in the abdomen which may become infected, spider-like blood vessels on the skin. Other complications include hepatic encephalopathy, bleeding from dilated veins in the esophagus or dilated stomach veins, and liver cancer. Hepatic encephalopathy results in confusion and may lead to unconsciousness². According to Lancet 2016, Liver cirrhosis (LC) is most commonly caused by alcohol, hepatitis B, hepatitis C, and non-alcoholic fatty liver disease. Typically, more than two or three alcoholic drinks per day over a number of years is required for alcoholic cirrhosis to occur. Non-alcoholic fatty liver disease has a number of causes, including being overweight, diabetes, high blood fats, and high blood pressure. A number of less common causes of cirrhosis include autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, certain medications, and gallstones. Diagnosis is based on blood testing, medical imaging, and liver biopsy Hepatocellular carcinoma (HCC) is a primary liver cancer that is more common in people with cirrhosis. People with known cirrhosis are often screened intermittently for early signs of this tumor, and screening has been shown to improve outcomes⁴. Hepatic cancer is a cancer that originates in the liver, which malignant tumors that grow on the surface or inside the liver. They are formed from either the liver itself or from structures within the liver, including blood vessels or the bile duct. It is the fifth most frequently diagnosed cancer worldwide and the third leading cause of cancer death ^{5, 6}. Because liver cancer is an umbrella term for many types of cancer, the signs and symptoms depend on what type of cancer is present. Cholangiocarcinoma is associated with sweating, jaundice, abdominal pain, weight loss and hepatomegaly⁷. Hepatocellular carcinoma is associated with abdominal mass, abdominal pain, emesis, anemia, back pain, jaundice, itching, weight loss and fever ⁸. Risk factors of HCC that were identified include hepatitis B virus (HBV), hepatitis C virus (HCV), cirrhosis, heavy alcohol consumption, non-alcoholic steatohepatitis (NASH), alfatoxin exposure, Obesity, increasing age, male sex, and positive family history ⁹. Diabetes mellitus (DM), commonly referred to as diabetes, which is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced ¹⁰. People with type 2 diabetes often develop a condition called "fatty liver". In these cases, the liver has trouble handling the abundance of fat in its cells and gradually becomes inflamed. That situation can trigger a cascade of problems, including cirrhosis (a chronic disease of the liver), fibrosis (thickening and scaring of tissue) and, ultimately, cancer. Type 2 diabetics have twice the chance of having a fatty liver, at least. Type 2 diabetes is an increasingly common metabolic disorder strongly linked

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to obesity and characterized by hyperglycemia, with increased risk of several cancers ¹¹. Substantial evidence indicates that diabetes promotes the development and progression of HCC ¹². The association between diabetes and HCC has been further demonstrated by studies published from different geographical locations ¹³. This study was applied on voluntary Egyptian patients aged between 18-60 of a mixture of males and females. And the purpose of the study is to investigate the effect of diabetes on the liver damaged cells in both two phases of liver cirrhosis (LC) and the hepatocellular carcinoma through analyzing different biomarkers such as blood sugar, Alpha-fetoprotein (AFP) and liver function (alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, total bilirubin (TB) and direct bilirubin (DB).

EXPERIMENTAL

The experimental has been run on forty admitted patients at Tropical Medicine and Gastroenterology, Minia university hospital, Egypt. Furthermore, twenty clinically healthy subjects were included in the study as a healthy control group. All patients were eligible for the study.

Patients grouping: Patients were classified into three groups as following:

Group 1: as a control group: Twenty volunteer persons served as healthy subjects and they did not show any clinical or biochemical disorders.

Group 2: Twenty volunteer patients with proven liver cirrhosis (LC) associated with DM. [LC + DM].

Group 3: Twenty volunteer patients with proven hepatocellular carcinoma HCC & diabetes mellitus DM. [HCC + DM].

Sample collection: Venous blood sample was collected from each subject using a sterile plastic syringe. The blood was kept in tube and allowed to clot then centrifuged to separate the serum for the tests. All biochemical and hematological parameters were carried out in Minia university hospital lap, except cortisol hormone and Alfa Feto Protein (AFP) were done in Elkasr Eleni hospital lap. Previous history of patients has been considered with special emphasis on suggestive symptoms of liver cell failure (e.g. jaundice, ascites, lower limb edema) and the suggestive symptoms of portal hypertension (e.g. previous history of bleeding varicose). In addition, clinical examination with stress on the clinical signs of liver cirrhosis (firm sharp bordered liver), signs of liver cell failure (e.g. palmer erythema and flapping tremors), and signs of portal hypertension (e.g. ascites, splenomegaly, and lower limbs edema) has also been taken in consideration.

Inclusion criteria: Males & Females ages (18-60 years). Liver cirrhosis with diabetes mellitus patient [LC & DM] and hepatocellular carcinoma with diabetes mellitus patients [HCC & DM]. Exclusion criteria: Patients with hepatic encephalopathy. Patients with renal failure. Patients with serious complications in heart, kidneys, or lungs. Patients with autoimmune diseases such as autoimmune hepatitis.

Data and statistical figures

The Statistical Package for the Social Sciences (IBM SPSS for WINDOWS 7, version 20; SPSS Inc, Chicago) was used for the statistical analysis of the results. Comparative analysis was conducted by using the general linear models procedure (IBM SPSS). F-probability: p<0.001, p>0.05 were considered statistically non-significant, while p<0.05 were considered statistically significant.

Data are expressed as Mean \pm SE. Values in all tables with individual superscript such as xa, xb...etc are not significant in the SE, and the values share the same superscript symbol, such as xa,b or xb,c are not significantly different in SE.

RESULTS AND DISCUSSION

This study reveals a number of important findings on the relationship between the diabetic groups of hepatocellular carcinoma and liver cirrhosis, this study showed a significant increase in Random Blood Sugar in patients groups compared with the control group. Results in table 1 and the graph in Fig. 1 revealed that there is a significant increase in random blood sugar in liver cirrhosis with diabetes group than in HCC with diabetes group. This could be attributed to the metabolic homeostasis of glucose in chronic hepatic disease is impaired as a result of a disorder of different factors such as insulin resistance, glucose intolerance and diabetes. Our findings were in agreement with the results of other studies¹⁴.



Figure 1: Random blood sugar concentration among different groups.



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Table 1: Random blood sugar and Alpha-fetoproteinamong different groups.

Groups	RBS	AFP
Control	112.5 ± 4.78^{a}	$5.5 \pm 0.95^{\circ}$
liver cirrhosi& diabetes mellitus [LC + DM]	363.3 ± 29.5 ^c	12.46 ± 1.79 ^ª
Hepatocellular carcinoma/Diabetes mellitus [HCC/DM]	205 ± 0.68 ^b	1326.2 ± 454.82 ^c
LSD	19.92	212.53

The study showed also a significant increase in alfa feto protein (AFP) in hepatocellular carcinoma with diabetes mellitus compared with control group or cirrhotic groups with diabetes mellitus (table 1, Fig. 2). These findings are in agreement with other reported studies [15-18], which proved that AFP is a glycoprotein produced by the fetal liver, yolk sac, and the gastrointestinal tract. Also this elevation due to injury in the liver, and the duration hepatocytes respond by proliferation, producing AFP^{19, 20}.

Monitoring the liver function via watching the elevation of some significant enzymes such as alanine transferees enzyme (ALT) and aspartate transferees enzyme (AST) is very important as they considered as a widely biological markers used to investigate the liver diseases. The present investigation showed a significant increase in serum liver enzymes activity of ALT and AST (Table 2; Figs. 3, 4). These results are again in agreement with Life Science report 2013, which revealed that there was a significant increase in ALT, AST in patients with chronic liver disease due to damaged liver cells which might causing the increase of these enzyme activities.





These results are also in agreement with literature ²¹ that explained the elevation of serum enzymes is a result of an increase of hepatic cell membrane fluidity which leads to enzyme release into circulation.

Groups	ALT	AST	Alb	T.Bil	D.Bil
Control	31.06 ± 2.38^{a}	31.94 ± 1.44^{a}	$4.3 \pm 0.15^{\circ}$	0.67 ± 0.03^{a}	0.2 ±0.01 ^a
liver cirrhosi& diabetes mellitus [LC & DM]	69 ± 14.23 ^b	95.6 ± 16.91 ^b	2.2 ± 0.02^{b}	$2.32 \pm 0.34^{\circ}$	1.69 ± 0.23^{b}
Hepatocellular carcinoma/Diabetes mellitus [HCC & DM]	118.3 ± 17.31 ^c	163.9 ± 30.33 ^c	1.78 ± 0.03 ^a	3.65 ± 0.31 ^c	3.05 ± 0.33 ^c
LSD	17.3	24.86	0.11	0.5	0.37

Table 2: Liver function assessment of different groups.



Figure 3: Serum alanine aminotransferase (ALT) concentration among different groups.

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Figure 4: Serum aspartate aminotransferase (AST) concentration among different groups.

Table 2 and Figs. 5 & 6 showed also an increase in total bilirubin (TB) and direct bilirubin (DB) levels in the serum of hepatocellular carcinoma with diabetes mellitus group compared with liver cirrhosis with diabetes group, indicate impaired excretory and synthetic functions of the liver at end stages of the disease ²². Also cholestasis disease and hemolysis is due to a prototypical cholestasis

disorder ²³. Increased serum bilirubin level is a result of liver dysfunction and hyperbilirubinemia is a weak antioxidant defense mechanism by itself. An increasing body of experimental evidence incriminates oxidative stress as a pivotal signal for liver fibrogenesis according to Life Science, 2013 and ²⁴.



Figure 5: Serum Total Bilirubin (T.B) concentration among different groups.







Serum albumin is the most abundant plasma protein and is essential for maintaining oncotic pressure of the vascular system. It can be considered useful tool in clinical scores, for evaluating liver function. Albumin is produced by hepatocytes which are produced in the liver, so the injury resulted from liver disease could decrease the Serum albumin level [25]. Indeed, our results are in agreement with such facts, where the study showed a significant decrease in serum albumin in both phases of liver disease (see table 2, Fig. 7).





CONCLUSION

Glucose intolerance was the main reason behind increasing the level of random blood sugar in patients with liver cirrhosis & diabetes mellitus. Also diabetes mellitus is associated with about two to three fold increased risk of HCC and increasing the risk of death from HCC in the large cohort study.

Diabetes often develops a condition called "fatty liver". In these cases, the liver has a trouble in handling the abundance of fat in its cells and gradually becomes inflamed. That situation can trigger a cascade of problems, including cirrhosis (a chronic disease of the liver), fibrosis (thickening and scaring of tissue) and, ultimately, cancer HCC, which associated with increased mortality that appeared in all biochemical parameters.

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