Research Article

Diabetes Mellitus as a Risk Factor in Patients with Hepatocellular Carcinoma - Biochemical Studies - Part 2.

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ABSTRACT

In this study we report the effect of diabetic mellitus (DM) on patients with hepatocellular carcinoma (HCC) via monitoring various of biochemical markers such as blood sugar, Alpha-fetoprotein (AFP), cortisol hormone, liver function (alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, total bilirubin (TB), direct bilirubin (DB), renal function e.g. creatinine, urea and uric acid in addition to a complete blood picture e.g. total leukocytes count (TLC), serum platelets (PLT) and serum hemoglobin (HB). 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 hepatocellular carcinoma and group 3: as patient with hepatocellular carcinoma associated with diabetes mellitus (DM). Results showed that diabetes mellitus (DM) is associated with about two to three fold increased risk of HCC. It was also observed that diabetic mellitus was the main reason behind increasing the risk of death from HCC in the large cohort study.

Keywords: Hepatocellular carcinoma (HCC), Diabetes mellitus (DM), biochemical markers, liver disease.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a global problem, ranking as the fifth most common malignancy among men and women in the world with high morbidity and mortality[1,2]. HCC represents the major histological subtype of primary liver malignancies, accounting for 70% to 85% of the total liver cancer burden[3,4]. Although the incidence of HCC rises with increasing age, reaching its peak in those aged above 65 years and more commonly in men over the past two decades there has a shift in incidence towards a younger age[5]. Egypt has possibly the highest HCV prevalence in the world; 10%–20% of the general population is infected and HCV is the leading cause of HCC and chronic liver disease in the country. Approximately 90% of Egyptian HCV isolates belong to a single subtype 4a, which responds less successfully to interferon therapy than other subtypes[6]. The burden of HCC has been increased in Egypt with a doubling in the incidence rate in the past 10 years[7]. Globally, as of 2010, liver cancer resulted in 754,000 deaths, up from 460,000 in 1990, making it the third leading cause of cancer death after lung and stomach. Of these deaths 340,000 were secondary to hepatitis B, 196,000 were secondary to hepatitis C, and 150,000 were secondary to alcohol[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]. However, in 15%-50% of HCC patients no specific risk factor has been reported[10, 11]. Because liver cancer is an umbrella term for many types of cancer, the signs and symptoms depend on what type of cancer is present. Cholangio-carcinoma is associated with sweating, jaundice, abdominal pain, weight loss and hepatomegaly[12]. Hepatocellular carcinoma is associated with abdominal mass, abdominal pain, emesis, anemia, back pain, jaundice, itching, weight loss and fever[13].

Diabetes mellitus (DM) is a worldwide epidemic disease according to world health organization (WHO) and recent estimation in 2015 showed 415 million people had diabetes world-wide[14]. Diabetes mellitus if left untreated can cause acute complications include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death. Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes according to WHO 2016. DM is associated with about two to three fold increased risk of HCC and may also increase the risk of death from HCC, which has been observed in a large cohort study conducted in Europe. Recently, emerging evidence suggest that diabetes mellitus DM is a potential risk factor for HCC which has been strengthened by several meta-analyses according to Medicine Baltimore 2017 and[15]. 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. Fatty liver disease is the first cause of HCC. Type 2 diabetics have twice the chance of having a fatty liver[16,17]. Moreover, substantial evidence indicates that diabetes promotes the development and progression

of HCC[18]. The association between diabetes and HCC has been further demonstrated by studies published from different geographical locations[19]. Based on such findings, we get interested to study the effect of diabetes on HCC patients, particular in Egypt where HCV is highly spread.

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 hepatocellular carcinoma (HCC).

Group 3: Twenty volunteer patients with proven hepatocellular carcinoma & diabetes mellitus (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). Hepatocellular carcinoma patients and hepatocellular carcinoma with diabetes mellitus patients.

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 ± SE. Values in all tables with individual superscript such as $x^{a,}$ x^{b}…etc are not significant in the SE, and the values share the same superscript symbol, such as $x^{a,b}$ or $x^{b,c}$ are not significantly different in SE.

RESULTS AND DISCUSSION

This study reveals a number of important findings on the relationship between hepatocellular carcinoma and diabetes mellitus. It showed a significant increase in random blood sugar (RBS) hepatocellular carcinoma with diabetes mellitus patients compared with the control non-diabetic cases. Thus, the RBS in hepatocellular carcinoma group was 137.6 ± 7.7, but it showed a significant increase in hepatocellular carcinoma with diabetes mellitus group at 205 ± 0.68 compared to the control group at 112 ± 4.78. (See table 1; figure 1)

Table 1: Random blood sugar (RBS), alpha-fetoprotein (AFP) and cortisol concentrations in all experimental groups.

Groups	RBS	AFP	Cortisol
Control	112.5 ± 4.78^{a}	5.5 ± 0.95^{a}	9.16 ± 0.56^{a}
Hepatocellular carcinoma (HCC)	137.6 ± 7.7^{a}	753.8 ± 328.54^{b}	19.07 ± 1.58^{ab}
Hepatocellular carcinoma/Diabetes mellitus(HCC/DM)	205 ± 0.68^{b}	1326.2 ±454.82^{c}	42.7 ±2.37^{b}
LSD	19.92	212.53	2.71

RBS in control group is 112±4.78, in non-significance in hepatocellular carcinoma group is 137.6 ± 7.7, and in hepatocellular carcinoma with diabetes mellitus group is 205 ±0.68.

The results are in agreement with other studies which showed that the diabetes mellitus (DM) is associated with about two to three fold increasing of HCC risk which also could increase the risk of death[4, 15].

The results are also in agreement with the results of other studies which proved that DM-mediated HCC development could be mediated through inflammation, cellular proliferation, apoptosis inhibition, and generation of tumor-causing mutations[20-23]. This confirms also the association between the duration of DM and HCC risk is significant[20].

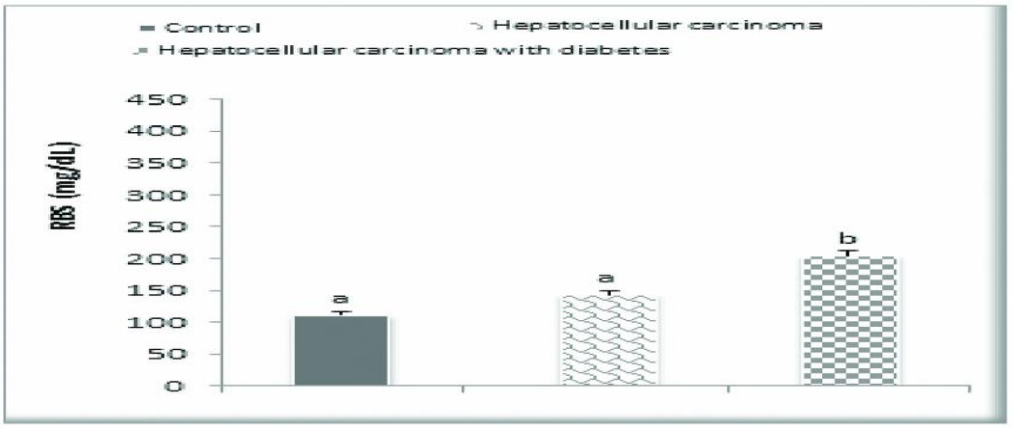

Figure 1: Random blood sugar concentration among different groups.

The single most important tumor marker for HCC is Alfa Feto Protien (AFP) in clinical practice. HCC surveillance with serum AFP level and ultrasonography has been recommended for patients with cirrhosis[24-26]. The present study showed a significant increase in AFP concentration in hepatocellular carcinoma with diabetes mellitus group and hepatocellular carcinoma group compared with control group. Thus; AFP level in control group was 5.5 ± 0.95 while a significant increase in hepatocellular carcinoma group was at 753.8 ± 328.54 and in hepatocellular carcinoma with diabetes mellitus group was 1326.2 ± 454.8. (See table 1; figure 2).

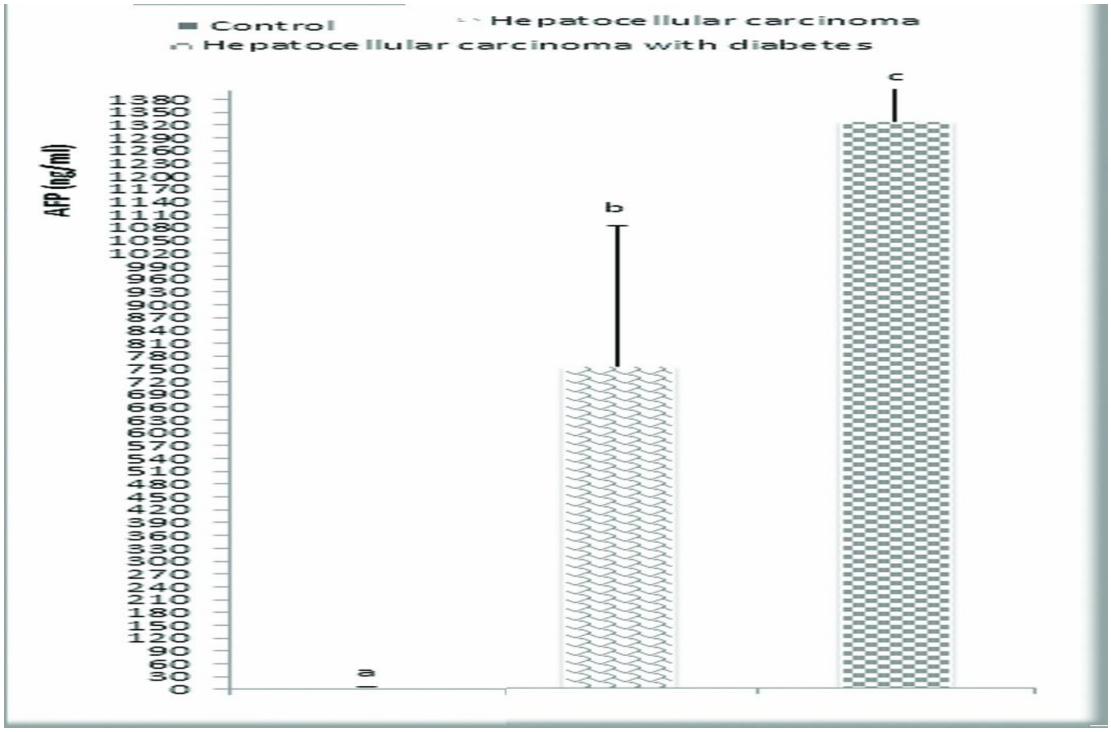

Figure 2: Alfa feto protein (AFP) concentration among different groups.

These results are in agreement with the results of other studies which proved that AFP is a glycoprotein which is normally produced by the fetal liver, yolk sac, and the gastrointestinal tract. Although it is most commonly elevated in HCC, also, elevations in serum AFP can be seen in various malignancies including testicular, bile duct, pancreatic, stomach, and colon cancer[5,27-29]. Note that this study showed a significant increase in AFP in hepatocellular carcinoma with diabetes mellitus group than hepatocellular carcinoma group due to injury in the liver, and the duration hepatocytes respond by proliferation, producing AFP[5], also the association between the duration of DM and HCC risk was significant in univariate analyses which directly increased AFP level[20,26].

Hypothalamuc -pituitary axis (HPA) secretes corticotropin releasing hormone (CRH) which affects adrenocorticotropic hormone (ACTH) that release from anterior pituitary gland. ACTH effects on adrenal cortex to secreted cortisol[27]. Liver is the primary site of adrenal steroid hormone metabolism and cholesterol synthesis[28]. Liver dysfunction may disrupt and lead to progressive impairment HPA activation during severe sepsis and septic shock which was proposed in hepato-adrenal

syndrome in patients with liver disease so it effects on cortisol level[29,31].

So this study confirmed that there is a significant increase in cortisol titers in all groups compared with control group due to HPA dysfunction in patients with liver disease[30]. Thus, cortisol titers in hepatocellular carcinoma with diabetes mellitus group was 42.7 ±2.37 but was moderate concentration in hepatocellular carcinoma group at 19.07 ± 1.58 compared to control group at 9.16 ± 0.56. (See table 1 & Figure 3). The mechanisms by which liver disease leads to HPA dysfunction have not been clarified, but some hypotheses have been suggested cholesterol is an essential precursor for steroid biosynthesis in adrenal glands. A decrease in total cholesterol, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol concentrations have been shown in cirrhosis related to the severity of liver disease[32]. This could lead to lack of substrates and to a progressive exhaustion of adrenal reserve (i.e., adrenal-exhaustion syndrome)[33].

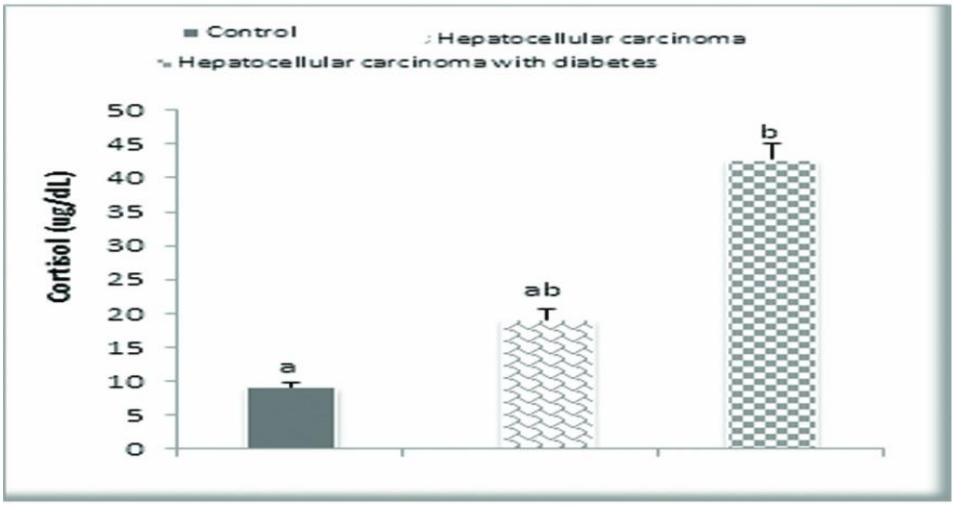

Figure 3: Cortisol concentration among different groups.

The cortisol titer in control group is 9.16 ± 0.56, in hepatocellular carcinoma with diabetes mellitus group is 42.7 ±2.37 and in hepatocellular carcinoma group is19.07 ± 1.58.

On other hand, alanine transferase enzyme (ALT) and aspartate transferase enzyme (AST) have been suggested for the two enzymes of greatest clinical significance in viral hepatitis and other forms of liver disease associated with hepatic necrosis[34].

The present investigation showed also a significant increase in serum liver enzymes activity such as alanine transfrase enzyme (ALT), aspartate transfrase enzyme (AST), serum, total bilirubin concentration and direct bilirubin concentration of all groups compared to the healthy group. (See table 2 & figures 4 for ALT).

Table 2: The Liver function bio-indicators among all groups.

Groups	ALT	AST	Alb	T.Bil	D.Bil
Control	31.06 ± 2.38^{a}	31.94 ± 1.44^{a}	4.3 ± 0.15^{d}	0.67 ± 0.03^{a}	0.2 ±0.01 ^{a}
Hepatocellular carcinoma (HCC)	64.1 ± 11.41^{ab}	103.3 ± 16.36^{b}	2 ± 0.03^{ab}	2.65 ± 0.31^{bc}	1.29 ± 0.24 ^{c}
Hepatocellular carcinoma/Diabetes mellitus(HCC/DM)	118.3 ± 17.31^{c}	163.9 ± 30.33^{c}	1.78 ± 0.03^{a}	2.99 ± 0.59^{c}	3.05 ± 0.33 ^{abc}
LSD	17.3	24.86	0.11	0.5	0.37

Serum aminotransferase (ALT) in control group is 31.1±2.38, in hepatocellular carcinoma group 64.1 ±11.41, and in hepatocellular carcinoma with diabetes mellitus group is 118.3 ± 17.31.

Thus, table 2 showed a significant increase in ALT level in hepatocellular carcinoma with diabetes mellitus (HCC + DM) and hepatocellular carcinoma (HCC) groups at 118.3 ± 17.31 and 64.1 ±11.41 respectively compared to the control group at 31.1±2.38. Similarly, AST level in hepatocellular carcinoma with diabetes mellitus and hepatocellular carcinoma groups was observed on high levels at 163.9 ± 30.33 and 103.3 ± 16.36 respectively compared to the control group at 31.94 ± 1.44. This enzyme elevation might be attributed to the increase in the hepatic cell membrane fluidity which could lead to extra enzyme release into the serum circulation[35,36]. It is obvious, that the effect of diabetes mellitus is the reason behind the jump in both ALT and AST levels from 118.3 ± 17.31 and 163.9 ± 30.33 in HCC + DM group to 64.1 ±11.41 and 103.3 ± 16.36 in HCC patients.

Total and direct bilirubin (TB and DB) levels in blood can also become important biomarkers that can help in

diagnosing the specific liver problems. In this study, the results showed a dramatic increase in both of total and direct bilirubin in hepatocellular carcinoma with diabetes mellitus group at 2.99 ± 0.59 (for TB) and 3.05 ± 0.33 (for DB) compared to the healthy group at 0.67 ± 0.03 and 0.2 ±0.01 respectively (see table 2). The increased serum bilirubin level could be a result of liver dysfunction and hyperbilirubinemia according to the reported study[37,38] rather than hemolysis due to liver dysfunction[39].

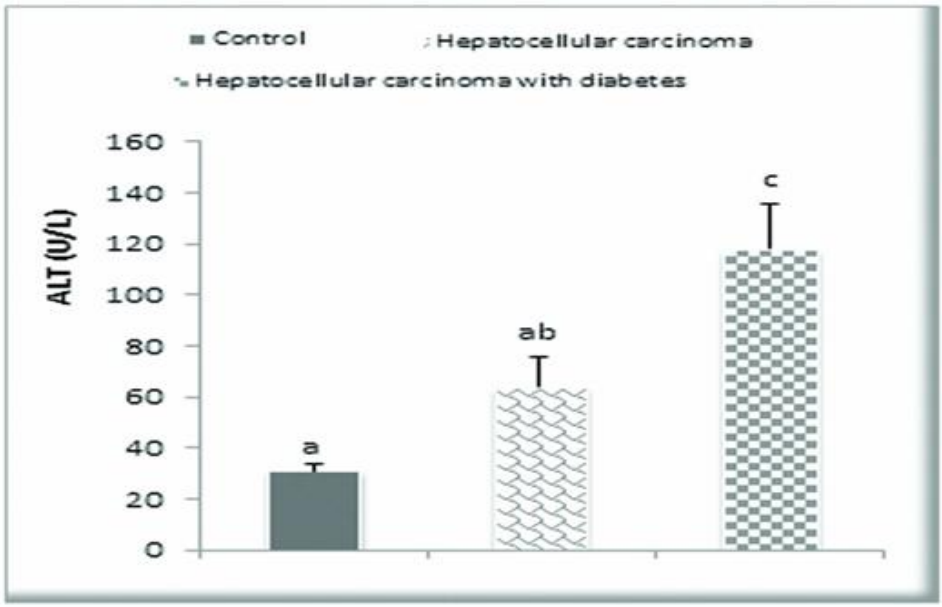

Figure 4: Serum alanine aminotransferase (ALT) concentration among different groups.

The serum albumin is the most abundant plasma protein and is essential for maintaining oncotic pressure of the vascular system. Because albumin is produced by hepatocytes which are produced in the liver, so it was considered as a useful tool in clinical scores to evaluate liver function. So the injury resulted from liver disease decrease the serum albumin level[40]. As an agreement with these reports, the present investigation showed a significant decrease in albumin in hepatocellular carcinoma with diabetes mellitus group (1.78 ± 0.03) compared to the control group (4.3 ± 0.15) while hepatocellular carcinoma group 2 ± 0.03 as shown in table 2.

The present results showed also a significant decrease in white blood cells count, hemoglobin (HB) concentration and platelets count in all groups compared to the healthy group. Results in table 3 showed that, platelets count has been decreased to 36000 ± 11171.64 in HCC group and 35000 ± 8865.97 in HCC+MD patients compared to the healthy group (485000 ± 28722.81) (figure 10). Similarly, HB showed a significant decrease in HCC + DM group (9.8 ± 0.33), and 10.29 ± 0.48 compared to the healthy patients (14.25 ± 0.47) (Table 3). The decrease in the hematological parameters indicates an immune disturbance due to the liver disease. It was reported that the HCC is associated with anemia and leucopenia in most patients[41]. There are various possible explanations, which include increased sequestration and destruction of platelets in patients with hypersplenism, reduced thrombopoiesis as a result of decreased production of endogenous thrombopoietin by diseased liver. Direct marrow suppression by hepatitis C virus as well as dysregulation of immunity leading to autoimmune thrombocytopenia.

Table 3: Complete blood picture among different groups.

Groups	TLc	Platelets	HB
Control	6450 ± 2.23^{a}	485000 ± 28722.81^{b}	14.25 ± 0.47^{b}
Hepatocellular carcinoma (HCC)	12950 ± 995.53^{c}	36000 ± 11171.64^{a}	10.29 ± 0.48^{a}
Hepatocellular carcinoma/Diabetes mellitus(HCC/DM)	14430 ± 614.36^{c}	35000 ± 8865.97^{a}	9.8 ± 0.33^{a}
LSD	2151.55	24913.46	0.89

The present study also revealed that, HCC and HCC& DM patients exhibited a notable increase in renal function e.g. serum urea, creatinine and uric acid concentration compared to the healthy group (see table 4). Where urea is a byproduct from protein breakdown and about 90% of urea produced is excreted through the kidney[42]. Moreover, the creatinine is a waste product from a muscle creatinine, which is used during muscle contraction, commonly measured as an index of glomerular function and it is excreted exclusively through

the kidney. Therefore, damage to the kidney will make the kidney inefficient to excrete both urea and creatinine and causes their accumulation in the blood. Therefore, the high level of blood urea and creatinine will indicate kidney damage[43]. Our results are in consistent with Dalrymple *et al*. 2007[44] who found that liver disease was associated with an increased prevalence of renal insufficiency. Beside chronic liver disease, relevant extrahepatic manifestations of HCV infection include renal disease[45].

Table 4: Complete blood picture among different groups.

Groups	Creat	Urea	Uric A
Control	0.95 ± 0.095[a]	28.5 ± 2.87[a]	4.75 ± 0.478[bc]
Hepatocellular carcinoma (HCC)	2.23 ± 0.03[b]	44.3 ± 8.43[a]	6.4± 0.37[c]
Hepatocellular carcinoma/Diabetes mellitus(HCC/DM)	2.4 ± 0.122[b]	65.7 ± 10.63[b]	7.3 ± 0.32[d]
LSD	0.136	10.06	0.34

Moreover, HCC may result in renal insufficiency through its effect on the liver where cirrhosis is associated with alterations in renal blood flow and decreases the immune complex clearance which could resulti a in renal injury[46]. In addition, HCC produces more reactive oxygen species (ROS) than other hepatitis viruses which subsequently led to renal insufficiency[37].

CONCLUSION

It is clear that diabetes mellitus (DM) is a major risk factor in liver disease particularly in HCC patient as indicated in all results. It is also could increase the risk of death in such cases. Diabetes often develops 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 HCC, which associated with increased mortality that appeared in all biochemical parameters

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