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Original Research Article

Hepatocellular Carcinoma and Its Association with Diabetes and Obesity

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Abstract: This investigation examined the relationship between hepatocellular cancer and diabetes and obesity. Twenty-eight male rats at age 2-3months were used in this study, were divided into 5 groups: G1 control negative fed normal feed, G2 Fed concentrated feed till obesity, G3 STZ was given once at 70 mg/kg B.W for the induction of DM and fed normal feed and G4 STZ was given once at 70 mg/kg B.W for the induction of DM and fed concentrated feed. Blood was aspirated weekly for serum separation. The glucose and liver function tests were done at 0,3,6,12 weeks of the study. Livers were collected from rats at end of experiment for histopathology and immunohistochemical detection of GS expression in hepatocytes. The current results showed that glucose levels increase significantly ($p \leq 0.05$) in G3 and G4 as compared with control group, while G2 showed increase in glucose levels with the time advanced. The levels of both AST and ALT were increased significantly in G3 and G4 when compared with G1, while G2 showed increasing both parameters with days advancement. One rat from G2 showed HCC, while G3 showed that 3 animals were have HCC and G4 showed that 4 animals with HCC. These liver tumors histologically resembled human HCC in terms of portal invasion and severe nuclear and structural atypia. Groups of liver tumors all responded well to the recognized human HCC marker GS. In conclusion, the results of this research provide support to the hypothesis that diabetes and obesity both have a role in elevating the risk of HCC.

Keywords: Obesity, Diabetes, liver, HCC, DM, GS.

INTRODUCTION

More than a million additional cases of hepatocellular carcinoma are anticipated worldwide by 2025, making it the most common kind of primary liver cancer [1]. Numerous conditions, including as hepatitis virus infection, alcohol abuse, NAFLD, as well as dietary toxins (such as aflatoxins), may result in HCC [2]. Genetic and epigenetic factors including DNA methylation and the mutation of programmed cell death-1 may both hasten the development of HCC [3].

NAFLD is the leading cause of HCC in the West, especially the United States [4]. NAFLD and severe NASH are associated with obesity, T2DM as well as cardiovascular disease [5].

Obesity, cardiovascular disease, type 2 diabetes, NAFLD, as well as colorectal cancer are all diseases in which the gut microbiota and its linked factors, such as metabolites and components, play crucial roles in the pathogenesis [6–9]. Fatty liver, fibromatosis, nonalcoholic steatohepatitis, as well as HCC are among conditions that may develop in rats fed an HFHC diet [10].

In the group of diseases linked to obesity, HCC has a modest risk. Increased levels of lipoteichoic acid (LTA) have been connected to dysbiosis of the gut microbiota, which in turn has been associated with obesity [12]. By encouraging the proliferation of senescent hepatic stellate cells (HSCs), LTA, a component of the membranes of Gram-positive bacteria, may promote the development of HCC [12]. Deoxycholic acid (DCA), a secondary bile acid produced by gut microbiota, and LTA cooperate to upregulate the expression of SASP factors and cyclooxygenase-2 (COX2) through Toll-like receptor

2 (TLR2), leading to COX2-mediated prostaglandin E2 (PGE2) production and a reduction in antitumor immunity [12, 13].

This investigation examined the relationship between hepatocellular cancer and diabetes and obesity.

Experimental

MATERIALS AND METHODS

Twenty-eight male rats at age 2-3months were used in this study, were divided into 5 groups:
G1 control negative fed normal feed
G2 Fed concentrated feed till obesity
G3 STZ was given once at 70 mg/kg B.W for the induction of DM and fed normal feed
G4 STZ was given once at 70 mg/kg B.W for the induction of DM and fed concentrated feed

Intraperitoneal injections were used for rats that had fasted overnight, and all treated rats were given access to food and water. Fasting blood glucose levels in rats were also measured after four days of stability. The study employed rats with glycemia levels more than 17 mmol/L as a diabetic model [14].

Blood was aspirated weekly for serum separation. The glucose and liver function tests were done at 0,3,6,12 weeks of the study. These kits were purchased from Biolabo Company and works done according to manufacturer instructions.

Liver tumors were examined grossly by slicing whole livers at 2-mm intervals after fixing them in 10% neutral buffered formalin. Paraffin was used to embed all sections with tumors that could be seen grossly, and these sections were then thinned down to 4 m for morphological and immunohistochemical analysis under the microscope. Histological sections were examined under a microscope after being deparaffinized, stained with hematoxylin and eosin, dehydrated in 100% ethanol, cleared with xylene, and mounted.

Primary antibodies against glutamine synthetase (Abcam, UK), sal-like protein 4 (Abcam, UK), and epithelial cell adhesion molecule (Abcam, UK) were used for immunohistochemistry. Antigen retrieval and blockage of endogenous peroxidase with 5% hydrogen peroxide in methanol for 5 minutes at room temperature were performed on the specimens after deparaffinization (RT). After blocking nonspecific binding with 5% bovine serum albumin, the samples were treated with prediluted primary antibodies at 4 degrees Celsius for 24 hours. Following application of EnVision Polymer-Hrp, the sample was incubated for 1 hour at 4 degrees Celsius. DAB was used to see the immunoreaction, and hematoxylin was used as a bright counterstain.

RESULTS AND DISCUSSIONS

The current results showed that glucose levels increase significantly ($p \leq 0.05$) in G3 and G4 as compared with control group, while G2 showed increase in glucose levels with the time advanced (Table 1).

Table 1: Glucose levels (mmol/l) at different times of study (Mean ± SE)

Groups	Zero day	3 wk	6 wk	12 wk
G1	5.3±0.4Ab	5.5±0.1Ac	5.24±0.03Ac	5.32±0.01Ac
G2	5.4±0.03Ab	8.1±0.3Bb	9.3±0.8Bb	9.8±0.03Bb
G3	15.21±0.51Aa	15.8±0.02Aa	16.2±0.02Aa	15.7±1.5Aa
G4	15.1±0.06Aa	16.15±0.02Aa	18.6±0.12Ba	18±1.2Ba

Capital letter mean differences between groups Small letters mean differences between time

In the STZ-induced diabetes model, the beta cells acquire the drug through a glucose transporter mechanism. Reportedly, STZ inhibits the enzyme aconitase, leading to DNA alkylation. This is achieved by the release of high quantities of nitric oxide and nitrosourea [15]. The prevalence of insulin resistance in STZ animal models is affected by a variety of factors, including but not limited to, animal age, STZ dosage, and animal strain. Experiments with HFDs were initially described in the 1940s, when rats given an extremely high-fat diet (consisting of 70% of their daily calories from fat) became obese and had raised baseline and postprandial blood glucose levels [16, 17].

The levels of both AST and ALT were increased significantly in G3 and G4 when compared with G1, while G2 showed increasing both parameters with days advancement (Table 2).

Table 2: Liver function tests in different groups at different days of study

Groups	AST (U/L)				ALT (U/L)			
	Zero day	3wk	6wk	12wk	Zero day	3wk	6wk	12wk
G1	44.1±1.9Ab	44.8±0.7Ab	46.2±3.4Ac	47.2±1.5Ac	4.3±0.01Ab	4.6±0.7Ac	4.4±0.02Ac	4.4±0.1Ac
G2	45.6±1.1Aa	52.6±0.7Ba	65.1±3.7Ca	67.8±7.1Ca	4.5±0.01Aa	5.7±0.02Ba	6.9±0.1Ca	7.4±0.05Ca
G3	65.1±1.7Aa	66.2±1.2Aa	67.9±2.4Ab	66.8±0.7Ab	7.2±0.03Aa	6.95±0.02Ab	7.13±0.09Ab	7.21±0.02Ab
G4	66.3±2.1Aa	64.5±0.3Aa	69.2±2.0Bb	72.1±0.9Bb	7.35±0.011Aa	7.47±0.2Ab	7.94±0.03Bb	8.6±0.03Cc

Capital letter mean differences between groups Small letters mean differences between time

These enzymes are often transferred to the plasma as a result of hepatocyte injury. Increased plasma triglyceride and cholesterol levels were seen in rats given a high-concentrated diet, which finally led to the development of lipotoxicity and lipid buildup in the liver [18]. Steatosis results from the buildup of fat in the liver, which is referred to as non-alcoholic fatty liver disease (NAFLD). To explain how a high-fat diet causes the development of NAFLD, a "two hit" approach has been put forth [18, 19]. The contributing variables and key elements of this "two hit" theory [20], include inflammation, insulin resistance, fat storage, and oxidative damage. Rats given a high-fat diet in the current research showed lipid buildup and an increase in inflammatory cell infiltration in the liver. Additionally, rats on a high-fat diet had enhanced myeloperoxidase activity, which is a component of invading cells, mostly neutrophils. Probiotics are thought to be helpful in reducing liver and intestinal inflammation [21, 22].

One rat from G2 showed HCC, while G3 showed that 3 animals were have HCC and G4 showed that 4 animals with HCC (Fig. 1). These liver tumors histologically resembled human HCC in terms of portal invasion and severe nuclear and structural atypia. Groups of liver tumors all responded well to the recognized human HCC marker GS (Fig. 2).

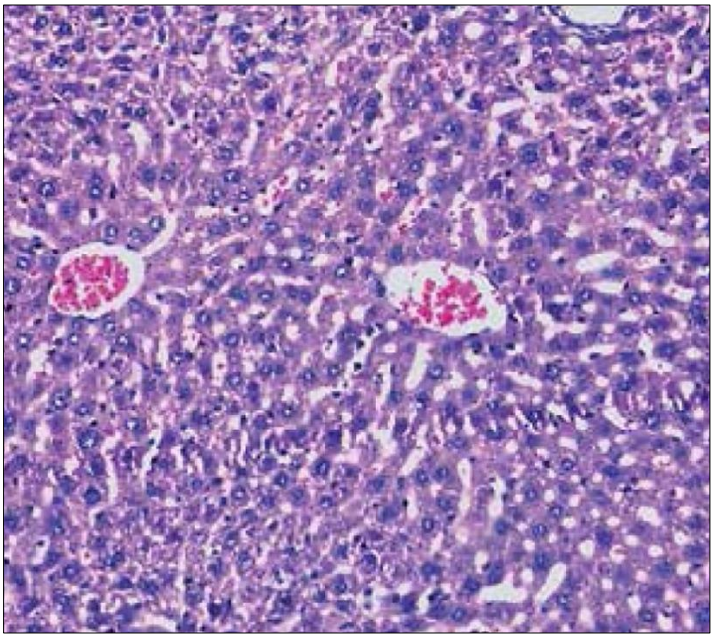

Figure 1: The solid sheets of cancerous cells that made up the liver tissue exhibited pleomorphism, elevated nucleo/cytoplasmic ratio, and mild to moderate nuclear atypia (H&E, 400X)

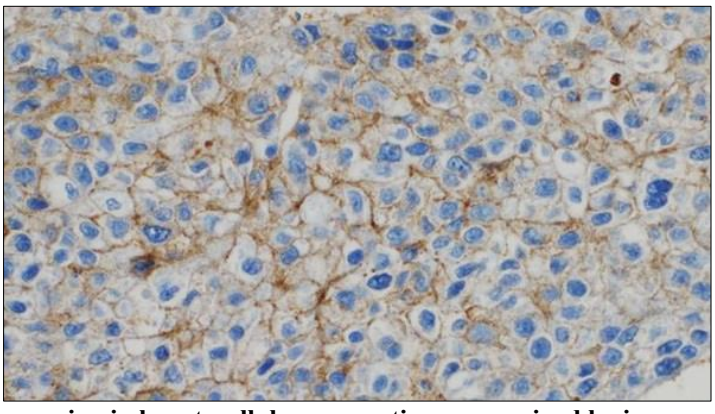

Figure 2: GS expression in hepatocellular cancer tissues examined by immunohistochemistry

Metabolic diseases are frequent in obese and diabetic people, and high liver fat buildup may lead to hepatocellular damage. These individuals are quite likely to have nonalcoholic fatty liver disease (NAFLD), and being overweight is linked to an advanced illness [23].

Although not statistically significant, the relationship between HCC risk and obesity was in line with OR estimates found in previous meta-analyses of cohort studies [24]. Obese people had higher HCC risks, according to studies on certain subgroups [25]. Likewise, cohort [26–28], and case–control studies [29], revealed a two–fourfold higher incidence of HCC among diabetics. Most of these investigations were unable to adequately demonstrate a temporal link, i.e., whether DM preceded HCC and any other liver illness predisposing to HCC. Cirrhosis [30, 31], and hepatitis infection [32], two diseases that result in varying degrees of liver failure, are frequent in patients with DM. However, DM was a risk factor for HCC in the current analysis as well as in a different case-control investigation [33].

CONCLUSION

The results of this research provide support to the hypothesis that diabetes and obesity both have a role in elevating the risk of HCC.

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