Association of Insulin Resistance, Diabetes Duration and Diabetes Treatment with Risk For Hepatocellular Carcinoma

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Abstract: Diabetes mellitus has been put forward as a potential risk factor for hepatocellular carcinoma (HCC) by some studies; however, no consensus has been reached about the true role of diabetes mellitus (DM) in HCC. The aim of the present study was to elucidate the role of insulin resistance (IR), diabetes mellitus and serum adiponectin level in the risk of HCC. Subjects and methods: A total of 288 subjects selected from Menoufiya and Al Azhar University's Hospital were divided into three groups. First group: 111 newly diagnosed patients with HCC. Second group: 97 patients with liver cirrhosis (LC). Third group: 80 apparently healthy subjects as a control group. All individuals included in this study were subjected to full history taking and clinical examination. Serum insulin, HBs Ag, Anti-HCV, insulin resistance (IR) assessed by homeostatic model (HOMA–IR) and serum adiponectin. Results: This study showed that there were 80 patients (73.1%) had type-2 DM in HCC group. While only 36 patients (37.1%) in LC group had type-2 DM compared to 23 (28.8%) subjects in control group. Also this study found a higher statistical significance increase of serum adiponectin level in HCC group than LC and control groups. In conclusion, the present study provides further evidence that insulin resistance and diabetes mellitus increase the risk of HCC. Also, serum adiponectin level seems to play a role in the development HCC.
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Key words: Diabetes mellitus, insulin resistance, adiponectin, cirrhosis, hepatocellular carcinoma.

1. Introduction

Hepatocellular carcinoma (HCC) is a significant worldwide health problem with as many as 500,000 new cases diagnosed each year. There is a considerable geographical variation in the incidence of HCC (**Parkin, 2006**). In Egypt, HCC is third among cancers in men with >8000 new cases predicted by 2012 (**Mizokami, 2005; Lehman and Wilson, 2009**). The HCC epidemic in Egypt is associated with hepatitis C viral (HCV) infection. Egypt has the highest prevalence of HCV in the world with ~13.8% of the population infected and seven million with chronic HCV liver disease (**Perz and Alter, 2006**). Up to 90% of HCC cases in the Egyptian population were attributed to HCV (**Hassan *et al.*, 2001**).

The natural progression of HCV infection to hepatitis, cirrhosis and HCC is slow. Chronic hepatitis develops in ~80% of those infected with HCV. Over the course of ≥20 years, 10–30% of HCV carriers develop cirrhosis; patients with cirrhosis have an annual risk of 1–2% for developing HCC (**Ikeda *et al.*, 1998**). The prognosis of patients with HCC remains extremely poor. The currently available systemic therapies demonstrate poor to modest response rates and have not been shown to improve survival in patients with HCC (**Liovet, 2005**). The slow development and late detection of HCC suggest that the identification of biomarkers of disease progression and early detection represents attractive strategies for potential improvement of the outcome of HCC patients (**Liovet, 2005**).

The WHO has reported HBV to be second only to tobacco as a known human carcinogen (**Parkin *et al.*, 1999**). Many studies on HCC risk following chronic HBV infection have been conducted in the East Asian countries, where most patients acquired HBV as newborn infants (**Montalto *et al.*, 2002; Kawaguchi *et al.*, 2004**). The incidence of HCC in HBV-related cirrhosis in this area of the world has been reported to be 2.7% (**Yeh *et al.*, 2007**). The annual risk of HCC is 0.5% for asymptomatic HBs Ag carriers and 0.8% for patients with chronic hepatitis B (**Bosch *et al.*, 2004**). While patients with HBV-cirrhosis have 1000 times higher risk of developing HCC, compared to HBs Ag negative individuals. Thus, it is likely that the probability of acquiring HCC increases with severity of underlying liver disease (**Michielsen *et al.*, 2005**).

A population-based study from the USA found diabetes to be an independent risk factor for HCC, regardless of chronic HCV or HBV infection, alcoholic liver disease, or non-specific cirrhosis. Diabetes was associated with a two- to threefold increase in HCC risk (**Kawaguchi *et al.*, 2005**). **Kawaguchi *et al.*, 2005** stated that about 60% of

patients with HCC were not diagnosed with chronic HCV-related or HBV-related hepatitis, alcoholic liver disease, or other known causes of chronic liver disease. Among these patients, 47% had diabetes, which was higher than those with other risk factors (41%). The suggests that diabetes may represent a considerable proportion of patients with idiopathic HCC.

Insulin resistance is frequently seen in patients with hepatitis C virus (HCV) infection (**Davila *et al.*, 2005**). Although in the general population, lack of exercise and overeating are major causes of insulin resistance, in patients with HCV infection, hepatic inflammation, activated inflammatory cytokines, and HCV-induced impairments of insulin and lipid signaling molecules are also important factors for the development of insulin resistance (**Tazawa *et al.*, 2002**).Therefore, the prevalence of insulin resistance is higher in patients with HCV infection compared to that in the general population and patients with other hepatobiliary disorders (**Franz *et al.*, 2002; Nagao and Sata, 2009**).

Generally, insulin resistance results in the development of type 2 diabetes mellitus and increases the risk of life-threatening complications such as cardiovascular diseases, renal failure, and infections. However, these complications are not major causes of death in cirrhotic patients with insulin resistance (**Franz *et al.*, 2002**). On the other hand, the development of intrahepatic complications, including hepatocellular carcinoma (HCC), is known to be associated with insulin resistance (**Duckworth *et al.*, 2009**). Insulin resistance is also reported to be involved in the development of extrahepatic manifestations of HCV infection including gastric cancer (**Qamar *et al.*, 2009**). Reduction of fasting blood glucose and glycated hemoglobin (HbA1c) is a well-established therapeutic strategy for prevention of complications in diabetic patients (**Itou *et al.*, 2008; Donadon *et al.*, 2009**). However, in patients with chronic liver diseases, fasting blood glucose and HbA1c are not always available for evaluation of glucose metabolism because of decreased hepatic glycogen content and increased turnover of hemoglobin (**Kawaguchi *et al.*, 2009**). Furthermore, an association between the use of exogenous insulin or sulfonylurea agents and the development of HCC has recently been reported (**Eguchi *et al.*, 2009**).

Aim of the Study:

The aim of the present study was to elucidate the role of insulin resistance (IR), glycemic control (determined by glycated hemoglobin), antidiabetic treatment and serum adiponectin level in risk of HCC.

2. Material and Methods:

Patients

First group:111 newly diagnosed patients with HCC from outpatient cancer clinic of National Liver Institute, Menoufiya University and El Azhar outpatient cancer cilinic (80 male and 31 female; mean age: 55.9 ± 7.8 years). The diagnosis of HCC was based on typical HCC feature on a dynamic image and alpha-fetoprotein (AFP) > 400 ng/ml.

Of these patients, 19 patients (17.1%) were positive for hepatitis B surface antigen (HBsAg), 55 patients (49.5%) were positive for anti-HCV antibody, 9 patients (8.1%) were positive for both HBsAg and anti-HCV, and 28 patients (25.2%) were negative for both HBsAg and anti-HCV.

Second group: 97 patients with liver cirrhosis (LC) (75 male and 22 female, mean age: 54.2 ± 6.2 years) which diagnosed by percutaneous liver biopsies according to the modified Knodell histological activity index. Of these patients, 6 patients (6.2%) were positive for HBsAg, 70 patients (72.2%) were positive for anti-HCV antibody, 9 patients were positive for both HBsAg and anti-HCV and 12 patients (12.4%) were negative for HBs Ag and anti-HCV.

Third group: 80 apparently healthy subjects as a control group were selected matched for HCC and LC patients according to age (mean age: 53.8± 6.2) and gender (58 male and 22 female).

A written informed consent was obtained from all participants. The study was approved by the local ethical committee in university hospitals.

Clinical and laboratory assessments

- Patients with a body mass index (BMI) of 18.5-24.9 kg/m^2 were classified as normal, 25-29.9 kg/m^2 as overweight and ≥ 30 kg/m^2 obese. The diagnosis of type 2 DM was based on the American Diabetes Association revised criteria, using a value of fasting blood glucose of ≥ 126 mg/dL on at least two occasions or ongoing treatment with hypoglycemic agents.
- Each patient was questioned about prior history of diabetes mellitus, the type of diabetes (insulin treated or non-insulin treated), the age at diagnosis and duration of diabetes.
- Subjects with history of diabetes were questioned about medication used for treatment and duration of treatment.
- Venous blood sample were collected before any treatment for tumor and taken in the morning after 12 hour overnight fasting. Blood samples were available for plasma glucose & HbAIc and stored serum samples for insulin level & adiponectin.
- Fasting blood glucose level was measured using enzymatic colorimetric glucose oxidase method (Kit purchased from Spin react, Spain).

- Blood sample from cases and controls were tested for HBV surface antigen (HBsAg) using Kits from Sorin-Biomedica Co. (Italy) and anti bodies against HCV (anti –HCV) using third – generation enzyme link immunoassay using Murix kits (Republic of South Africa). These kits implement qualitative methods based on enzyme linked immunosorbant assays (ELISA). Procedures were done according to manufacturer's instructions.
- Glycated hemoglobin was measured by quantitative turbidimetric technique using Vital Diagnostic, Italy.
- Serum insulin levels were done using radioimmunoassay (Coat-A-Count insulin kit; Diagnostic Products Corp., Los Angeles, CA, USA).
- Insulin resistance was calculated by using homeostasis model assessment (HOMA-IR) score that employs the formula: fasting insulin concentration (uIU/ml) × glucose (mmol/l) / 22.5 (**Matthews *et al.*, 1985**). Measurement of glucose level by mg/dl was multiplied by 0.555 to get result by mmol/l to calculate HOMA-IR.
- Circulating level of adiponectin was measured by sandwich ELISA using commercial kits according to the manufacturer's instructions (Quantikine ELISA kits; R&D Systems, Inc., Minneapolis, MN, USA) (**Kishore and Reid, 2000**).

Statistical Analysis:

All the data were collected and entered to the computer using SPSS (Statistical Package for Social Science) program for statistical analysis. Data were presented as mean ± SD. The Pearson`s correlation coefficients were calculated for the normally distributed values. However, Spearman`s correlation coefficients were done for the not normally distributed values. Continuous normally distributed variables were tested for association by student's t-test. On the other hand, Mann–Whitney *U* test or z test is a non-parametric test for assessing whether two independent samples of observations have equally large values. P value <0.05 was considered statistically significant.

3. Results:

Table (1) illustrates the mean levels of BMI, AST, ALT, platelets, cholesterol, triglyceride, fasting blood glucose, HbA1c, insulin, HOMA-IR and adiponectin between HCC, LC and control groups. Table (2) showed among HCC group, there were 80 patients (72.1%) had type-2 DM. While only 36 patients (37.1%) in LC group had type-2 DM compared to 23 (28.8%) subjects in control group. In HCC group, there were 55 patients (49.5%) having Anti-HCV, 19 patients (17.1%) were positive for HBsAg, 9 patients were positive for both Anti-HCV and HBsAg and 28 patients (25.2%) were negative for both Anti-HCV and HBsAg. When compared the diabetic patients in the three groups, table (3) showed that there wasn't a statistical significant relation between them regarding the age of onset and the type of the treatment of the diabetes mellitus.

Using a correlation in overall groups, this study showed positive correlations between adiponectin and each of mean serum level of cholesterol, triglyceride, fasting blood glucose, HbA1c and HOMA-IR (r=0.245, 0.169, 0.322, 0.288 and 0.248, respectively) (Table 4). Regarding the relationship between the tumor size and type-2 DM in HCC patients, no statistical significant relationship was found between them as in table 5. Table (6) showed no statistically significant relationship was found between tumor size and each of age of onset, duration and treatment of the type-2 DM and glycemic control of the patients.

Table (1): Statistical comparison of the different studied parameters in the three groups.

	Studied groups			P value
	HCC group N = 111 Mean ± SD	LC group N = 97 Mean ± SD	Control group N = 80 Mean ± SD	
BMI (kg/m^2)	25.45±5.78	20.08 ± 4.29	18.57 ± 2.66	<0.001
AST (U/L)	63.51±51.10	53.08 ± 32.84	29.48 ± 9.13	<0.001
ALT (U/L)	77.87±53.19	70.45± 48.46	38.31±13.21	<0.001
Platelets	159.46±110.87	199.65±105.24	261.52±71.45	<0.001
Cholesterol (mg/dl)	281.63±126.59	203.32±62.53	158.67±53.31	<0.001
Triglyceride (mg/dl)	268.88±198.92	177.09±73.63	140.0 ± 51.81	<0.001
Fasting blood glucose (mg/dl)	215.57±134.1	145.67±91.29	134.96±93.21	<0.001
HbA1c (%)	6.76±1.6	6.32 ±1.52	6.04 ± 1.72	<0.001
S. insulin (uIU/ml)	17.80 ± 3.38	13.64 ± 4.26	14.35 ± 4.01	<0.001
HOMA- IR	6.0 ± 5.87	3.17 ± 4.06	3.39 ± 3.46	<0.001
Adiponectin (ug/ml)	16.57±9.27	12.42 ± 5.97	11.04 ± 5.16	<0.001

BMI: body mass index, AST: aspartate aminotransferase, ALT: aspartate aminotransferase, HbA1c: glycated hemoglobin and HOMA-IR: homeostasis model assessment P <0.001 is highly significant

Table (2): Clinical characteristic in the three studied groups.

	Studied groups						P value
	HCC group N = 111		LC group N = 97		Control group N =80		
	NO	%	NO	%	NO	%	
Type-2 DM:							
Positive	80	(72.1)	36	(37.1)	23	(28.8)	<0.001
Negative	31	(27.9)	61	(62.9)	57	(71.2)	
Hepatitis :							
HBsAg & Anti-HCV: -ve	28	(25.2)	12	(12.4)	63	(78.7)	<0.001
HBsAg: + ve	19	(17.1)	6	(6.2)	5	(6.3)	
Anti-HCV: +ve	55	(49.5)	70	(72.2)	12	(15)	
HBsAg & Anti-HCV: +ve	9	(8.1)	9	(9.2)	0	(0.0)	
Child score:							
A	50	(45.0)	69	(71.1)	80	(100)	<0.001
B	25	(22.5)	22	(22.7)	0	(0.0)	
C	36	(32.4)	6	(6.2)	0	(0.0)	

P <0.001 is highly significant

Table (3): Statistical comparison of different studied parameters in the diabetic patients of the three studied groups.

	Studied groups						P value
	HCC group N = 80		LC group N = 36		Control group N =23		
	NO	%	NO	%	NO	%	
Age of onset :							
<50 years	52	(65.0)	25	(69.4)	16	(69.6)	0.05
> 50 years	28	(35.0)	11	(30.6)	7	(30.4)	
Duration of the disease:							
<2 years	26	(32.5)	3	(8.3)	7	(30.4)	<0.001
2 – 5 years	35	(43.8)	13	(36.1)	13	(56.5)	
6 – 10 years	12	(15.0)	1	(2.8)	0	(0.0)	
> 10 years	7	(8.8)	19	(52.8)	3	(13.1)	
Treatment :							
No ttt	3	(3.8)	0	(0.0)	0	(0.0)	
Diet therapy	4	(5.0)	1	(2.8)	2	(8.7)	0.05
Insulin	16	(20.0)	12	(33.3)	8	(34.8)	
Metformin	41	(51.3)	21	(58.3)	11	(47.8)	
Sulfa	16	(20.0)	2	(5.6)	2	(8.7)	

P 0.05 is non significant and P<0.001 is highly significant

Table (4): Correlation between adiponectin and different measured parameters in total groups:

	Adiponectin (ug/ml)	
	Correlation coefficient (r)	P value
BMI (kg/m^2)	+0.15	<0.01
Cholesterol (mg/dl)	+ 0.245	<0.001
Triglyceride (mg/dl)	+ 0.169	<0.001
Fasting blood glucose (mg/dl)	+ 0.322	<0.001
HbA1c (%)	+ 0.288	<0.001
Insulin (uIU/ml)	+ 0.080	0.05
HOMA-IR	+ 0.248	<0.001

P 0.05 is non significant and P<0.01 is highly significant

Table (5): Relationship between tumor size and type-2 DM in HCC group.

	Tumor size								P value
	< 3 cm N = 24		3 – 5 cm N = 72		> 5 cm N = 15		Total N=111		
	NO	%	NO	%	NO	%	NO	%	
Type-2 DM :									
Positive	14	(17.5)	52	(65.0)	14	(17.5)	80	(72.1)	0.05
Negative	10	(32.3)	20	(64.5)	1	(3.2)	31	(27.9)	

P 0.05 is non significant

Table (6): Relationship between tumor size and different variables in diabetic patients of HCC group

	Tumor size								P value
	< 3 cm N = 14		3 – 5 cm N = 52		> 5 cm N = 14		Total N = 80		
	NO	%	NO	%	NO	%	NO	%	
Age of onset:									
<50 years	9	(17.3)	35	(67.3)	8	(15.0)	52	(65.0)	□0.05
> 50 years	5	(17.9)	17	(60.7)	6	(21.4)	28	(35.0)	
Duration of the disease:									
<2 years	2	(7.7)	21	(80.8)	3	(11.5)	26	(32.4)	
2 – 5 years	9	(25.7)	20	(57.1)	6	(17.1)	35	(43.8)	□0.05
6 – 10 years	1	(8.3)	9	(75.0)	2	(16.7)	12	(15.0)	
> 10 years	2	(28.6)	2	(28.6)	3	(42.9)	7	(8.8)	
Treatment :									
No ttt	1	(33.3)	2	(66.7)	0	(0.0)	3	(3.8)	
Diet therapy	1	(25.0)	3	(75.0)	0	(0.0)	4	(5.0)	
Insulin	2	(12.5)	11	(68.8)	3	(18.3)	16	(20.0)	□0.05
Metformin	10	(24.4)	24	(58.5)	7	(17.1)	41	(51.3)	
Sulfa	0	(0.0)	12	(75.0)	4	(25.0)	16	(20.0)	
Glycemic control :									
Controlled	3	(15.0)	16	(80.0)	1	(5.0)	20	(25.0)	□0.05
Uncontrolled	11	(18.3)	36	(60.0)	13	(21.7)	60	(75.0)	

P□ 0.05 is non significant

4. Discussion:

Hepatocellular carcinoma (HCC) is a world wide malignancy, and the incidence rate has increased significantly over the past two decades in China, Japan, the USA and other countries (**Gao and Yao, 2009**). The reason for this increase in HCC has not yet been explained clearly, although more than 50 % of this increase has been attributed to hepatitis virus or alcoholic liver disease, especially to hepatitis virus (**Yuen *et al.*, 2009**).

Diabetes mellitus (DM) has been put forward as a potential risk factor for HCC by some studies; however, no consensus has been reached about the " true " role of DM itself directly predisposes to HCC (**Goa and Yao, 2009**).

Earlier epidemiologic studies showed no association between HCC and DM (**Lu *et al.*, 1988**), but some studies have identified DM as a risk factor for HCC (**Davila *et al.*, 2005**), especially two cohort studies conducted in Sweden and the USA (**El-Serag *et al.*, 2004**).

The aim of the current study was to elucidate the role of insulin resistance, glycemic control and serum adiponectin level in the risk of HCC.

The present study found higher statistically significance increase of diabetes among HCC group than LC and control groups. A total of 111 HCC patients (72.1%), 97 LC patients (37.1%) and 80 controls (28.8%) recalled a prior history of diabetes mellitus that conferred a 3–fold increase in HCC risk when compared with nondiabetic individuals (P = < 0.001).

These findings are in agreement with results obtained by **Manal *et al.* (2010)** who stated that diabetes appears to increase the risk of HCC, and such risk is correlated with a long duration of diabetes.

Our result in agreement with **Polesel *et al.* (2009)** who stated that obesity and diabetes increase HCC risk and that these factors may explain a relevant proportion of cases among subjects without markers of HBV / HCV infection.

The results of the current study are consistent with the notion that the biological mechanism for liver-cell damage induced by type 2 diabetes mellitus involves insulin resistance and hyperinsulinemia (**Harrison, 2006**). HCC development related to hyperinsulinemia can be mediated through inflammation, cellular proliferation, inhibition of apoptosis, and mutation of tumor suppressor genes (**Harrison, 2006**). Increased insulin levels lead to reduced liver synthesis and blood levels of insulin growth factor - binding protein - 1, which may contribute to increased bioavailability of insulin-like growth factor–1 (IGF–1), the promotion of cellular proliferation, and the inhibition of apoptosis (**Moore *et al.*, 1998**).

Our study revealed that patients with HCC had higher fasting blood glucose, serum insulin level and HOMA-IR than those with LC and control groups.

These findings are in agreement with results obtained by **Chao-Hung *et al.*(2010)** who demonstrated the independent association between insulin resistance and HCC development in chronic HCV infection.

Studying the role of adiponectin in HCC may be more complex because of its underlying chronic hepatitis infection (**Hang *et al.*, 2009**). Previous studies have demonstrated that circulating adiponectin levels are inversely associated with the

risk of malignancies associated with insulin resistance, including endometrial, breast, colon and gastric cancer (**Ishikawa *et al.*, 2005**). Moreover, serum adiponectin level has been reported to be significantly elevated in chronic liver disease and correlated with stage of liver cirrhosis, liver cell injury, e.g. aminotrasferase activity, and inflammatory markers (**Kaser *et al.*, 2005**).

Thus, serum adiponectin level is modified according to the two opposing factors, insulin resistance and underlying liver condition.

In this study, we found high significance difference in serum adiponectin level among the three groups with the highest serum adiponectin level in HCC patients. This result in disagreement with (**Chao-Hung *et al.*, 2010**) who found no difference in serum adiponectin level among different groups. This disagreement may explained on basis of difference in the nature and number of control subjects.

This study showed positive correlations between serum adiponectin level and each of mean serum level of cholesterol, fasting blood glucose , HbA1c and HOMA–IR.

In this study, we found no statistically significant relationship between tumor size and each of age of onset, duration and treatment of the diabetes mellitus and glycemic control of the patients.

This result in disagreement with (**Manal *et al.*, 2010**) who stated that diabetes appears to increase the risk of HCC, and such risk is correlated with a long duration of diabetes. Relying on dietary control and treatment with sulfonylurea or insulin were found to confer the highest magnitude of HCC rick, whereas treatment with biguanides was associated with a 70% HCC risk reduction among diabetics.

In conclusion, the present study provides further evidence that insulin resistance and diabetes mellitus increase the risk of HCC and serum adiponectin level seems to play a role in the development of HCC.

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