RESEARCH ARTICLE

Resistin and Insulin Resistance: A Link Between Inflammation and Hepatocarcinogenesis

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Abstract

Background: Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third leading cause of cancer related death overall. The role of insulin resistance in the development of HCC associated with chronic HCV infection has not been established. Resistin is a polypeptide hormone belonging to the adipokine family which could contribute to tumorigenesis and angiogenesis. Our aim was to study serum resistin and insulin resistance as risk factors for HCC in HCV cirrhotic patients. Materials and Methods: This prospective case controlled study included 100 patients with HCV related liver cirrhosis and HCC, 100 patients with HCV related liver cirrhosis without HCC and 50 apparently healthy participants as controls. For all subjects, liver profile, serologic markers for viral hepatitis, lipid profile, alpha-fetoprotein level (AFP), homeostasis model assessment (HOMA) were examined along with resistin. Results: HCC patients had higher mean values of HOMA-IR and resistin than cirrhotic patients and the control subjects ($p<0.01$). HOMA and resistin were considered independent risk factors in development of HCC, those patients with resistin › 12 ng/ml and HOMA ›4 being 1.6 times more likely to have HCC. Conclusions: HOMA and serum resistin allow for early identification of patients with cirrhosiswho are at substantially increased risk of HCC. Recommendation: HOMA and serum resistin could represent novel markers to identify HCV cirrhotic patients at greater risk of development of HCC.

Keywords: Resistin - insulin resistance - HOMA IR - hepatocellular carcinoma

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Introduction

Hepatocellular carcinoma (HCC) is the sixth most common tumour and third most common cause of cancer related deaths worldwide. Understanding the risk factors for HCC development in patients infected with HCV is of great importance to help in elucidating novel modalities in management. The relationship between metabolic factors and chronic hepatitis C and HCC has become a rapidly growing topic (Eslam et al., 2011). HCV per se is now considered to be a special type of metabolic syndrome, however the role of insulin resistance (IR) in HCV related hepatocarcinogensis remains unclear Fartoux et al., 2005). Resistin is a polypeptide hormone belonging to adipokines. Resistin acts as intrahepatic cytokine exerting pro-inflammatory actions (Bertolani et al., 2006). Several studies have indicated that resistin may significantly influence the growth and proliferation of malignant cells (Housa et al., 2006).

Our aim was to study serum resistin and insulin resistance as a risk factors for HCC in HCV cirrhotic patients.

Materials and Methods

This prospective case controlled study was conducted at Ain Shams university hospital between January 2014 and January 2015 with 250 participants divided into three groups.The first group comprised 100 patients with HCV related liver cirrhosis and HCC. Liver cirrhosis was diagnosed on the basis of history, clinical examination, laboratory findings, and abdominal ultrasonography (US). HCC was diagnosed by abdominal (US), abdominal triphasic CT and serum AFP (Bota S et al., 2012). The second group comprised 100 patients with HCV related liver cirrhosis without HCC and A third group comprised 50 apparently healthy participants (non diabetic with normal liver) as control group.

Exclusion criteria included: Concurrent human immunodeficiency virus infection, HBV, active alcohol consumption, previous history of treatment with interferon ther-apy for HCV, any treatment for HCC, current treatment with any dosage of insulin therapy, treatment with corticosteroids or any medications known to affect glucose tolerance or insulin secretion. This study was conducted in accordance with interna-tional ethical guidelines .Written informed consent was obtained from all participants prior to enrolment in the study.

All subjects were submitted to: *i)* Detailed history and physical characteristics with focus on stigmata of liver dis-ease. BMI was calculated as body weight in kilograms divided by the square of height in meters (kg/m^2). *ii)*

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Table 1. Demographic Data of Hepatocellular Carcinoma, Liver Cirrhosis and Healthy Control Groups

	HCC	LC	CONTROLOS	1 VS 2	1 VS 3	2 VS 3
age	52.3±6.2	52.2±8	51±7	>0.05	>0.05	>0.05
male	85	66	24	<0.01	<0.01	<0.05
female	15	34	26	<0.01	<0.01	<0.05
BMI	32.9±8.9	34.2±8.4	31.3±7	>0.05	>0.05	>0.05

Table 2. Laboratory Data of Hepatocellular Carcinoma, Liver Cirrhosis and Healthy Control Groups

	HCC	LC	CONTROLOS	1 VS 2	1 VS 3	2 VS 3
AST	66.6±22.7	65±22	18.7±5.6	>0.05	<0.01	<0.01
ALT	45.6±18.5	44±15.7	28±8	>0.05	<0.01	<0.01
BILIR	3.9±3	3.7±2.8	0.7±0.2	>0.05	<0.01	<0.01
ALBUMIN	2.4±0.5	2.4±0.5	4.3±0.5	>0.05	<0.01	<0.01
INR	2.5±0.7	2±1	0,8±0.1	>0.05	<0.01	<0.01
PLAT	79,2±35.3	71±35.4	273±75	>0.05	<0.01	<0.01
CHOLESTEROL	107±37	111,5±42	116.8±47	>0.05	>0.05	>0.05
LDL	106±35	108.9±43	112±45	>0.05	>0.05	>0.05
HDL	42.9±12.5	45.5±10	47.5±12	>0.05	>0.05	>0.05
TG	129.4±43	119.9±42	116.9±39	>0.05	>0.05	>0.05
FBS	5.1±0.9	4.2±0.7	3.8±0.5	<0.01	<0.01	<0.05
INSUIN	23.9±17	19.7±9.9	8±4.28	<0.05	<0.01	<0.01
HOMA	4.8±3.9	3.6±1.9	1.8±1	<0.01	<0.01	<0.01
RESISTIN	16.2±4	6.9 ±1.4	3.4±1.1	<0.01	<0.01	<0.01
AFP	296±635	28.4±51	3.6±0.8	<0.01	<0.01	>0.05

Biochemical assays: Haematological and biochemical workup included: CBC, re-nal profile, liver profile (PT, AST, ALT, Total and direct bilirubin, S albumin) for each patient, a modified Child-Pugh score was calculated. (Donadon et al., 2009) Serological tests: HBsAg, anti-HBc, HCVAb ,HCV PCR, α- Fetoprotein (AFP). Lipid profile (total cholesterol, HDL, LDL, TG). Fasting and 2h PP blood glucose, HA1C, fasting plasma insulin. Insulin resistance was assessed by the Homeostasis Model Assessment method (HOMA). The HOMA index of insulin resistance (HOMA-IR) was cal-culated as follows: (fasting insulin mU/L) × (fasting glucose mmol/L)/22.5. (Hui-Qi Qu, et al., 2011).Serum level of Resistin was measured using the Quantikine Human Resistin Immunoassay ELISA kit (Cat. No: DRSN00, Europe, United King-dom). *iii)* Radiological investigations: Ultrasound Doppler: Abdominal ultrasound us-ing real time scanning device Toshiba, vision 200 (SSA, 320A) with convex probe 3.5-5 uHz. The following details were recorded: Liver span ; surface nodularity; fo-cal lesion (no, site, size, echogensity), spleen size; diameter of the portal and splenic veins and presence of ascites.

Triphasic contrast enhanced computed tomography scan of the abdomen to confirm HCC. The characteristic radiological features means obvious hyper vascularization after application of contrast medium. Hyper vascularization characterized by con-trast enhancement in the early arterial phase rapidly disappears in the late venous phase (Bota et al., 2012).

Statistical analysis: All the collected data were expressed as mean ± SD and analyzed by using SPSS version 13 using the following tests: Chi square, ANOVA, Perason correlation coefficient and logistic regression. P>0.05 was considered non signifi-cant, P < 0.05 was considered significant P < 0.01 was considered highly significant.

Table 3. Correlation between HOMA and Resistin and Different Variables

	HOMA HCC	HOMA LC	RESISTIN HCC	RSISTIN LC
BMI	<0.01	<0.01	<0.05	<0.05
FBG	<0.01	<0.01	>0.05	>0.05
INSULIN	<0.01	<	<0.05	<0.01
HOMA			<0.01	<0.01
RESISTIN	<0.01	<0.01		
CHOLESTOROL	>0.05	>0.05	>0.05	>0.05
LDL	<0.05	<0.05	>0.05	>0.05
HDL	>0.05	>0.05	>0.05	>0.05
TG	>0.05	>0.05	>0.05	>0.05
AFP	<0.01	<0.01	<0.01	<0.01
CHILD SCORE	<0.01	<0.01	<0.01	<0.01
TUMPR NO	<0.01		<0.01	
TUMOR SIZE	<0.01		<0.01	

Results

Demographic data are listed in Table 1 showed that ages of HCC patients (mean 52.3 years), cirrhotic patients (mean 52.2 years) and control group (mean 51 years; range 38-63 years) were closely comparable (P>0.05), while. Preponderance of males was observed with both HCC (85%) and cirrhotic groups (66%). No significant difference was observed in BMI between the studied groups (p>0.05).

Table 2 showed biochemical characteristics: HCC patients showed significantly higher mean values of ALT, total bilirubin, direct bilirubin, and AFP than controls (P<0.01). HCC patients showed significantly higher mean values of AFP, FBG, fasting insulin, HOMA-IR and resistin than cirrhotic patients and the control subjects (p<0.01).

In cirrhotic and HCC patients there was significant positive correlations were found between HOMA-IR as

well as resistin and BMI, fasting insulin, Child score, AFP, tumor number and size as shown in Table 3

Based on stepwise logistic regression analysis HOMA and resistin were considered independent risk factors in development of HCC, patients with resistin >12 ng/ml and HOMA >4 were 1.6 times more susceptible to have HCC

Discussion

A multiplicity of viral and host factors may play a crucial role in facilitating the onset of IR in patients with chronic hepatitis C (CHC) that may ultimately end with HCC development (Cowey et al., 2006). Our results showed that HCC patients showed significantly higher HOMA-IR than cirrhotic patients and the control sub-jects ($p<0.01$). These findings were consistent with a previously published that IR is associated with high risk of HCC development in patients with chronic HCV (Gomaa et al., 2010; Hung et al., 2010 and Nkontchouemail et al., 2010; Eslam et al., 2011) reported that islets of pancreas in patients with cirrhosis show higher proliferation and lower apoptosis, compared to those in patients with no chronic liver disease, these finding suggest that hyperinsulinemia in cirrhotic patients may be caused by an adaptive response of the pancreatic beta cells to in-creased insulin resistance. It has been also suggested that increased levels of pro-in-flammatory cytokines such as interleukin 1, TNF-α, IL-6 and leptin, and reduced lev-els of adiponectin may directly contribute to the occurrence of HCV-related IR (Abdel-Rahman El-Zayadi and Mahmoud Anis, 2012).

HCV-associated insulin resistance is involved in the development of various compli-cations including, hepatic steatosis, resistance to anti-viral treatment, hepatic fibrosis and esophageal varices, hepatocarcinogenesis and proliferation of HCC, as well as extrahepatic manifestations (Jansson et al., 2010). This was in agreement with Donadon et al. whose study was conducted on 465 HCC patients, 618 with cirrhosis and 490 control subjects, they found that hyperinsulinemia is a characteristic features in all stages of the liver diseases and that the link between insulin and chronic liver disorders begins in the early stages of liver fibrosis and increases significantly when the liver disease advances towards cirrhosis and HCC. hyperinsulinemia which occur in IR can promote the synthesis and biological activity of insulin-like growth factor 1 (IGF 1), which is a peptide hormone that regulates energy-dependent growth process-es. IGF-I stimulates cell proliferation and inhibits apoptosis and has been shown to have strong mitogenic effects on a wide variety of cancer cell lines (Alexia et al., 2004). Excess insulin might affect the development of cancer indirectly by down regulating the level of IGF binding protein 1 which increases the level and bioavail-ablity of total circulating IGF1 showing the highest blood insulin levels, and this might have facilitated the development of HCC (Dailey, 2004). Insulin has a mi-togenic effect, through activation of a mitogen-activated protein kinase pathway, suggesting that insulin may be directly linked to hepatocarcinogenesis (Kawaguchi, 2010).

We found significant positive correlations between HOMA-IR and BMI, fasting insu-lin, FBG, LDL, child score and AFP in cirrhotic and HCC patients .

IR causes lipid accumulation, which results in changes in serum adipocytokine levels, including reduction of adiponectin, which has suppressive effects for hepatocarcino-genesis (Abdel-Rahman El-Zayadi and Mahmoud Anis, 2012). Hepatic lipid accumu-lation also increases oxidative stress, which may be responsible for the development of HCC (Takumi Kawaguchi., 2011). Insulin and HOMA-IR exhibited positive cor-relation with child score, tumour NO, size and AFP this finding may suggest inti-mate relation between metabolic disorder and HCV related HCC advanced hepatic fibrosis and disease severity results in more IR and impairs insulin clearance and vice versa (Hung, 2010). This was supported by Mohamed FS et al., who found that HOMA-IR was significantly higher in intermediate/advanced stage HCC pa-tients,compared to early stage HCC and HCV-positive cirrhotic patients respectively.

On the other hand, some authors reported conflicting results as they found that there is no association between IR and HCC(Amal A Mohamed et al., 2011; Mohamed AA et al., 2011 and Irshad M et al 2013). We found that resistin serum levels were sig-nificantly elevated in patients with liver cirrhosis compared with healthy controls and Resistin correlated significantly and positively with insulin ,HOMA and child score .

Yagmur et al., (2006) and Kakizaki et al., (2008) supported our results as they demonstrated that resistin increased with stage of liver cirrhosis as defined by Child-Pugh and re-sistin showed significantly positive correlation with fasting plasma insulin, HOMA-IR index. Binding of adiponectin to its receptors stimulates phosphorylation of PPAR activity and fatty acid oxidation in liver and reducing fatty acid synthesis through inhibition of acyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) ex-pression and activity (Martin et al., 2013), and this mechanism is inhibited by resistin. It is well known that inflammation is a key mechanism in the progression of fatty liver to hepatitis and cirrhosis (Diehl, 2002), therefore increased resistin may induce, steatosis, fibrosis via insulin resistance and inhibiting adiponectin ac-tion.

Serum resistin is proportionally related to cancer development, including: breast and colorectal cancers. It has also been suggested that the expression of resistin in cancer cells is associated with more malignant clinicopathological processes (Dalamaga et al., 2013). Our results showed that, HCC patients showed significantly higher mean value of serum resistin than cirrhotic patients and the control subjects, HOMA and resistin were considered independent risk factors in development of HCC, pa-tients with resistin > 12 ng/ml and HOMA >4 were 1.6 times more susceptible to have HCC. The interaction between intercellular adhesion molecule-1-(ICAM-1) and vas-cular cell adhesion molecule-1 (VCAM-1) and their respective ligand may facilitate the adhesion of cancer cells to the vascular endothelium, and subsequently aid in the promotion of metastasis. Resistin has been indicated to induce ICAM-1 and VCAM-1 expressions through transcription factor NF-ϰB in endothelial cells and to initiate the cancer cells and monocyte adhesion (Hsu et al., 2011), these effects were

attenu-ated by AMPK activation more over resistin has an inhibitory effect of adenosine monophosphate kinase activation, so resistin may play an important role to promote HCC metastasis (Yang et al., 2014).

In conclusions, HOMA and serum resistin allow for early identification of patients with CHC who are at substantially increased risk of HCC. These findings may have im-portant prognostic and therapeutic implications as IR is a potentially modifiable fac-tor.

Recommendation, HOMA and serum resistin could represent novel markers to identify the HCV cirrhotic patients at greater risk for the development of HCC. treatment of IR if present is recommended in patients with CHC

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