STUDY: THE CORRELATION BETWEEN HOMA-IR AND HEPATOCELLULAR CARCINOMA DEVELOPMENT IN HEPATITIS C PATIENTS

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ABSTRACT:

Background: Hepatocellular carcinoma (HCC) is the third most common cause of death from cancer worldwide which accounts for 80%-90% of primary liver cancer. It is characterized by a very poor prognosis. Outcome of HCC depends mainly on its early diagnosis. Serum α-fetoprotein (AFP) is the marker that has been widely used for screening and diagnosis of HCCs. However, development of false-negative or false-positive rates with (AFP) was as high as 30%-40% for patients with small HCCs. Insulin resistance (IR) is found to occur early in the course of Hepatitis C (HCV) infection, independent of BMI (body mass index), stage of liver disease and presence or absence of diabetes. Recently, it has been observed the synergistic effect of IR and viral hepatitis in HCC development among HCV infected patients. Therefore, this study was done to investigate the correlation between HOMA-IR and HCC patients. **Methods:** Clinical and biochemical characteristics were investigated for 50 HCC patients related HCV and 50 normal controls. HCC patients were diagnosed by ultrasound assessment, abdominal triphasic CT and serum AFP. Homeostasis model assessment of IR (HOMA-IR) was investigated to all 100 participants. **Results:** Obese patients in HCC group showed significantly higher frequency of high HOMA-IR when compared to non- obese patients (P=0.001). HOMA-IR value increases as tumor size of HCC increases.

Conclusion: Hepatocarcinogenisis may result from a combination of this direct viral effect and the influence of an array of metabolic factors resulting from virus-induced insulin resistance. **Key words**: HCV, HOMA-IR, HCC.

INTRODUCTION:

Hepatocellular carcinoma (HCC) is the most frequent cause of death in patients infected with hepatitis C virus (HCV), and epidemiological trends suggest that the mortality rate is rising **(Fattovich *et al.*, 2004)**. Egypt has the highest countrywide prevalence of HCV in the world since about 12 to 15% of the total populations are infected **(Zekri *et al.*, 2008)**. Therefore, understanding the risk factors for HCC development in patients infected with HCV is of great importance for treatment strategy. Significant attention is presently being drawn toward HCV as a cause of metabolic syndrome (includes: dyslipidemia, diabetes and insulin resistance (IR)) rather than simple viral infection. **Sheikh *et al.*, (2008)** summarized in their review the potential molecular pathways by which HCV contributes to IR. IR is a consistent finding in patients with type-2 diabetes mellitus (DM) and different lines of evidences have shown that patients infected with HCV have significantly higher IR than healthy controls matched for age, sex and body mass index (BMI) **(Hui *et al.*, 2003)**. Recent studies have reported that HCV-associated IR may cause 1) hepatic steatosis; 2) resistance to anti-viral treatment; 3) hepatic fibrosis and esophageal varices; 4) hepatocarcinogenesis and proliferation of HCC;

and 5) extrahepatic manifestations **(Kawaguchi & Sata 2010; Hung *et al.*, 2009).** A recent report has provided the first evidence that IR could increase the risk of developing HCC in patients with chronic HCV infection **(Hung *et al.*, 2010)**. Up to our knowledge this paper is the first one trying to correlate HOMA-IR and tumor size of HCC among Egyptian patients.

Patients and methods: This prospective study was conducted on 100 participants divided into two groups. Patients group included 50 samples of HCC patients related hepatitis C genotype- 4 virus. The other control group included 50 samples obtained from apparently healthy participants who had donated blood at the National Cancer Institute, Cairo University. A written consent was obtained from all patients prior to enrollment in the study and the ethical committee has approved the protocol, which was in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

Radiological study:

Abdominal ultrasonography was done for all patients**.**

Histopathological study: The liver biopsy specimens were collected intraoperative. Two specimens were obtained from every patient, one from the tumor tissue and the other from the surrounding non-tumor tissue. All specimens were fixed in formalin embedded then sectioned and stained by Haematoxylin & Eosin for routine histopathological examination to detect the criteria (grade and stage) of the disease. Histopathological grading and staging of chronic hepatitis was performed according to Modified Knodell's Score **(Ishak *et al.*, 1995).** They were graded according to the World Health Organization (**WHO**) classification criteria and staged according to the American Joint Committee on Cancer **(Hamilton & Aaltonen 2000)**.

Laboratory investigations: Venous blood samples were taken in the morning after 12-h overnight fast. Fasting glucose, HbA1C, serum alanine aminotransferase, aspartate aminotransferase, Gamma Glutamyle (GGT), albumin (Alb), total bilirubin levels (Bil), cholesterol (Chol), and triglyceride (TG) were measured by using synchron CX4- clinical system. Serum insulin levels and α-fetoprotein (AFP) levels were tested by serological technique using ELIZA technique according to manufacture instructions. Platelet count (Plt), Prothrombin Time (PT) and International Normalization Ratio (INR) were performed for all patients. IR was calculated on the basis of fasting levels of plasma glucose and serum insulin, according to the homeostasis model assessment (HOMA) method. The formula for the HOMA model is as follows: insulin resistance (HOMA-IR) = fasting glucose (mg/dL) × fasting insulin (μIU/mL)/405 **(Hong *et al.*, 2009).**

Statistical analysis: Data were analyzed using SPSS win statistical package version 15 (SPSS Inc., Chicago, IL). Chi-square test and Fisher's exact test were used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann-Whitney test. Comparison between 3 groups was done using ANOVA test or Kruskal-Wallis test followed post-Hoc "Schefe test". Spearman-rho method was used to test correlation between numerical variables. A p-value < 0.05 was considered significant.

RESULTS:

The total number of participants was 100 individuals, divided into two equal groups. Although the mean age was higher in HCC group than control group (58.8±9.66 versus 47.3±8.2), this difference was not statistically significant (P-value = 0.37). There was no statistically significant difference between both group as regard the gender, but preponderance of males was observed among HCC and control groups (1:1.6 and 1:1.06 respectively) (table 1). Biochemical parameters for both groups were also summarized in table (1) and showed the liver function tests (ALT, AST, T. Bil., Alb., INR and GGT) and platelets count of HCC

group which were appeared with statistically significant differences and elevations comparing with normal control group (P < 0.001). HOMA-IR and AFP in HCC group showed statistically higher significant differences comparing with normal control. HOMA-IR level in control cases was 0.77 within range 0.42 to 1.52 and HCC group was 4.18 ranges (0.91-32.2). There is no statistically significant difference between both group as regard the cholesterol and triglyceride level (table 1). The clinical data for HCC group was 42% with abdominal pain, 50% with jaundice, 90% with encephalopathy and 60% suffering from bleeding (table 2). Table (3) showed the correlation between HOMA-IR and tumor size of HCC patients , recording gradual increase (IR were 7.80 ± 3.66, 15.11 ± 4.11& 29.52 ± 2.82 in tumor size <3, 3-5 & >5 , respectively) with a statistically high significant correlation, while AFP recorded 12.52 ± 3.59, 47.71 ± 17.20 & 265.39 ± 127.11 in tumor size <3, 3-5 & >5, respectively . This study showed that obese patients in HCC group had a significantly higher frequency of high HOMA-IR when compared to non-obese patients (P=0.001) as in figure 1.

Table1. Demographical characteristics and metabolic factors among studied groups.

Variables	Normal control N=50	HCC N=50	**P-value**
Age (Mean ± SD)	47.3±8.2	58.8±9.66	0.37
Sex			
Male:	28	31	
Female:	22	19	
M:F ratio	1: 1.2	1:1.6	0.713
Obesity (>25kg/m^2)N(%)	18 (36%)	20 (40%)	0.725
Biochemical parameters			
Median (Range)	32 (20-47)	64(34-103)	0.092
ALT (IU/L)	36 (18-52)	120 (65-310)	**<0.001**
AST (IU/L)	0.92 (0.4-1.2)	2.2 (1.2-6)	**<0.001**
T. Bil (mg/dl)	4.2 (3.5-4.7)	3 (1.6-3.4)	**<0.001**
Albumin (g/dl)	0.91 (0.7-1.0)	1.2(1.1-1.5)	**<0.001**
INR ( )	37 (12-55)	190 (60-560)	**<0.001**
GGT (IU/L)			
F. Glucose (mg/dl)	100(80-147)	188(76-890)	**0.052**
F. Insulin (μIU/ ml)	4.1(2-6.1)	9(3.6-15)	**<0.001**
HbA1C (**%**)	4.3(2-7.2)	8(4.2-13)	**<0.002**
HOMA-IR	0.77(0.42-1.52)	4.18 (0.91-32.22)	0.043
Plt (x10^9/l)	350(144-465)	130(50-170)	**<0.001**
Cholesterol (mg/dl)	165(133-225)	190(110-300)	0.997
TG (mg/dl)	148(148-232)	190(120-300)	0.887
Tumor marker median(range)			
Serum AFP(ng/ml)	15.7 (2.9-22)	225 (150-1060)	**<0.001**

Data are median (range), frequency (%).
P -value: < 0.05 = statistically significant difference

Table 2: Clinical characteristics of HCC patients:

	No. of patients	**Percentage of patient**
Weight loss Yes	21	42%
Abdominal pain Yes	25	50%
Jaundice Yes	45	90%
Encephalopathy Yes	33	66%
Bleeding Yes	30	60%

Table 3: Serum HOMA-IR and AFP (ng /ml) levels in different tumor sizes in HCC group.

Parameters	**Tumor sizes**	**N**	**Mean ± SD**	**P-value**
HOMA-IR	<3	21	7.80 ± 3.66	P<0.001
	3-5	20	15.11 ± 4.11	
	>5	9	29.20 ± 2.82	
AFP (ng/ml)	<3	21	12.52 ± 3.59	P<0.001
	3-5	20	47.71 ± 17.20	
	>5	9	265.39 ± 127.11	

P value < 0.05 = significant

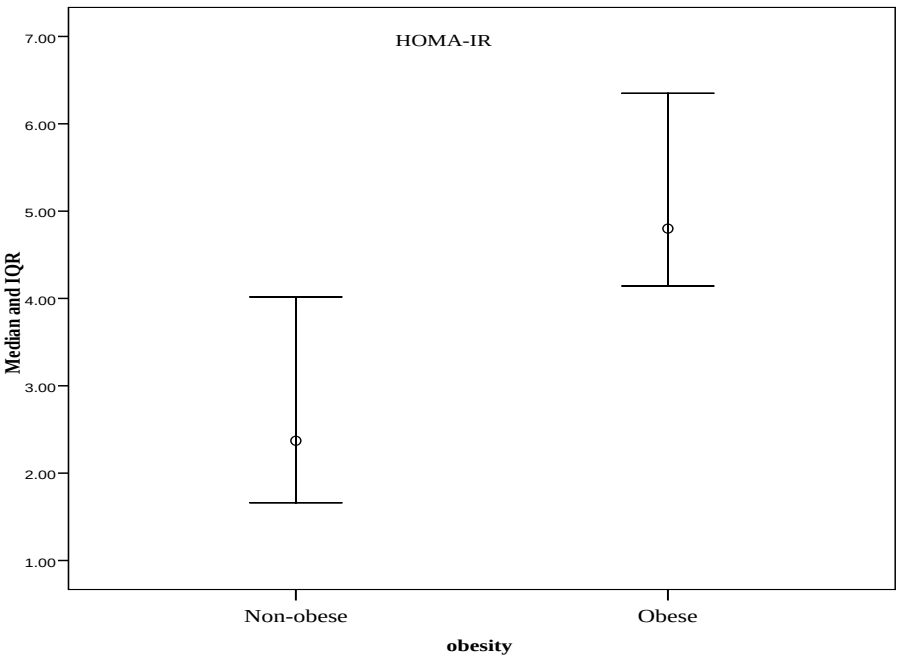

Figure 1: Plot of median and IQR of HOMA-IR among obese and non-obese in HCC group.

DISCUSSION:

Previous studies suggested a strong synergistic effect of metabolic factors and viral hepatitis in HCC development among HCV-infected patients **(Davilia *et al.*, 2005; Chen *et al.*, 2008)**. In contrast to the previous novel report which found the association of IR, regardless of diabetes with development of HCC **(Hung *et al.*, 2010; Lai *et al.*, 2006), Veldt *et al.* (2008)** founded that the 5 year risk of developing HCC is 11.4% for patients with both DM and HCC with advanced fibrosis. Patients without diabetes have lower risk of HCC with occurrence of HCC in 5% after 5 years. As regards obesity, it has been documented that presence of IR is associated with some medical conditions like: abdominal obesity, elevated cholesterol and hypertension **(Eckel *et al.*, 2005).**

In the present study, HCC patients were more common in males than females; that number of males were more than females, these results are similar to Zakhary et al. 2011 who reported that males represented 70.8% of all patients in HCC group, with 83.3% of patients over 50 years. In our study, we found that the mean value of AST, ALT , Bilirubin , INR and GGT were higher in HCC patients than that of the control group ,however albumin was lower in HCC group than that of the other group. These findings are in consistent with **Sun *et al.* (1998)** who reported that the previous parameters usually indicate the type of liver injury, whether hepatocellular or cholestasic but cannot be expected to differentiate one form of hepatitis from another or to determine whether cholestasis is intra or extra hepatic.

In the present study, obesity was found to be significantly associated with higher HOMA-IR level among obese HCC patients compared to non-obese patients ($P<0.001$). It has been reported that obesity may directly lead to a state of chronic inflammation that associated with an increase in the expression of several signaling molecules involved in the carcinogenesis process like NF-kB and fibroblast growth factor **(Nathan 2008; Bakwill & Mantovani 2001). Chen *et al.*, (2008)** have reported that although overweight itself did not increase risk of HCC to an important degree but when it was combined with diabetes, they showed a synergistic effect. Therefore, our results assumed a synergistic effect of obesity

when combined with high level of HOMA-IR in risk of HCC development associated HCV infection.

As regards host and viral factors, most of our HCC patients were above 57 years old. In univariate analysis, IR was correlated with age in HCV patients. It has been suggested that age is associated with a decline in mitochondrial function which could contribute to IR **(Petersen *et al.,* 2003).** However, this relation disappeared in multivariate analysis (Moucari et al., 2008). Moreover, several observations demonstrated that IR is an HCC risk factor in patients with chronic HCV. In the present study, there were correlated proportional increasing levels of HOMA-IR and AFP and tumor size of HCC group (table 3). These observations agree with **Kaji *et al.,* (2008)** who recently reported that IR itself significantly augmented vascular endothelial growth factor (VEGF)-mediated hepatic neovascularization and directly accelerate hepatocarcinogenesis. Our present observation and **Kaji *et al*. (2008)** study disagree with **Permert *et al.,* (1993)** that were documented that the insulin sensitivity was not correlated with weight loss, tumor size, or bilirubin level, but improved after surgery.

CONCLUSION:

This study indicates that in the Egyptian population suffering from a high burden of hepatitis C genotype- 4 virus, the strikingly high rates of hepatocarcinogenisis may result from a combination of this direct viral effect and the influence of an array of metabolic factors resulting from virus-induced insulin resistance.

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العلاقة بين مقاومة الانسولين و وجود سرطان الكبد في مرضى الالتهاب الكبدي سي

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يعد سرطان الكبد المسبب الثالث للوفاه الاكثر شيوعا بالعالم حيث انه المسئول عن 80-90 % من حالات سرطان الكبد الاوليه.ليس من السهل الكشف المبكر عن سرطان الكبد. تعتمد نتائج سرطان الكبد على الكشف المبكر عنه. يستخدم الفافيتو بروتين للكشف الاولى و كذلك لمتابعه سرطان الكبد , و مع ذلك نسبه الخطا لهذا الاختبار تعد عاليه تتراوح من 30-40% من اجمالى المرضى فى المراحل الاوليه.

وجد ان مقاومه الانسولين تحدث فى بدايه الاصابه بسرطان الكبد ,و ذلك بصرف النظر عن مقياس كتله الجسم (مقياس السمنه) , مرحله المرض الكبدى ووجود مرض السكري من عدمه.

لوحظ مؤخرا وجود علاقه تآزريه بين مقاومه الانسولين و الاتهاب الكبدى فى تطور سرطان الكبد لدى مرضى فيروس سي, لذا قامت هذه الدراسه بفحص العلاقه بين HOMA IR او مقاومه الانسولين و مرضى سرطان الكبد HCC.

طريقة البحث:

تم فحص الخصائص البيوكيميائيه و السريريه لخمسن مريض بسرطان الكبد الناتج عن فيروس سي و خمسين شخص طبيعى للمقارنه.

تم تشخيص سرطان الكبد عن طريق الموجات الفوق صوتيه و التصوير المقطعى لمنطقه البطن باشعه اكس و كذلك مستويات الفا فيتوبروتين بالدم .

كما تم قياس معدلات مقاومه الانسولين لكل من المائه شخص المشاركين .

النتائج:

اظهرت النتائج ان المرضى المصابين بالسمنه لديهم معدلات مقاومه للانسولين اكبر بشكل ملحوظ عن اولئك الغير مصابين بالسمنه.

و كذلك تبين ان معدلات مقاومه الانسولين HOMA IR تزداد بازدياد حجم الورم.

التوصيات:

نوصي بتطبيق البحث علي عدد اكبر من المرضي المصابين بالسمنه في المستقبل .