ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Insulin Resistance Among HCV Infected Liver Cirrhotic Patients With and Without Hepatocellular Carcinoma

Waleed Ahmady, Sherif M Galal, Talaat Fathy, Ehab Darwish, Ibrahim Mohammed
Tropical Medicine, Faculty of Medicine, Zagazig University, Egypt

Manuscript Info

Manuscript History:

Received: 17 August 2015
Final Accepted: 22 September 2015
Published Online: October 2015

Key words:

insulin resistance, HCV, cirrhosis, HCC

Corresponding Author

Waleed Ahmady

Abstract

Background: Hepatitis C virus infection is one of the most serious global health problems and major causes of chronic liver disease Egypt has the highest prevalence of HCV worldwide with approximately 20% of total population. HCV has been identified as a cause of metabolic syndrome that includes dyslipidemia diabetes and insulin resistance. HCV is the major risk factor for hepatocellular carcinoma development worldwide. **Aim of the work:** this study aimed at determining the frequency of insulin resistance in patients with chronic HCV induced liver cirrhosis and in patients with HCV-associated HCC. Insulin resistance in Egyptian patients infected with HCV genotype 4 has a role in predicting hepatocellular carcinoma. **Patients and methods:** 352 patients were included. Patients were divided into two groups. Group I included 176 patients; 120 males and 56 females with post-hepatitis C virus cirrhosis, Child grade A and B without HCC. Group II included 176 patients; 136 males and 40 females with post-hepatitis C virus cirrhosis, Child grade A and B with HCC. Fasting serum insulin level and homeostasis model assessment (HOMA) were estimated for all patients. Values were compared using appropriate statistical tests. **Results:** there was a highly significant statistical increase in group II compared with group I as regard fasting insulin (13.52±7.32 vs 10.9±6.107), HOMA-IR score(3.51±1.43 vs 2.72±1.14) and degree of IR (a total of 63:6% of patients of group II vs 44.3% of patients of group I with moderate and severe degrees of IR). **Conclusion:** IR may be a risk factor for development of HCC among HCV infected cirrhotic patients.

INTRODUCTION

Hepatitis C virus (HCV) infection is the leading cause of end-stage liver disease worldwide **[1]**.The overall prevalence of chronic HCV infection among world population according to WHO is 2.35% **[2]**. Egypt has the highest prevalence of HCV worldwide with approximately 20% of total population **[3]** and the highest prevalence of HCV of genotype 4 which is responsible for almost 90% of infections and is considered a major cause of chronic hepatitis, liver cirrhosis, hepatocellular carcinoma and liver transplantation in the country **[4]**.

The severity of the disease varies widely from asymptomatic chronic infection to cirrhosis and hepatocellular carcinoma **[5]**. HCV has been identified as a cause of metabolic syndrome; a complex that includes dyslipidemia, diabetes and insulin resistance (IR). IR is a key feature of this syndrome and a variety of potential molecular pathways by which HCV may contribute to IR have been suggested. Patients infected with HCV have significantly higher IR than healthy controls matched for age, sex and body mass index *[6]*.

Hepatocellular carcinoma (HCC) is the fifth most common malignancy in the world complicating liver cirrhosis in most cases **[7]**. Its incidence is increasing worldwide ranging between 3% and 9% annually **[8]**. In

Egypt, HCC was reported to account for about 4.7% of chronic liver disease (CLD) patients. The epidemiology of HCC is characterized by marked demographic and geographic variations **[9]**.

Recent studies have found that HCV-associated IR may cause hepatic steatosis, resistance to antiviral treatment, hepatic fibrosis, esophageal varices, hepatocarcinogenesis and proliferation of HCC and extrahepatic manifestations. IR has emerged as a risk factor for a wide variety of cancers. In chronic HCV infection, IR can favour fibrosis progression directly and act indirectly by inducing steatosis in a genotype-dependent manner. It has been reported that IR can increase the risk of developing HCC in patients with chronic HCV infection **[10]**.

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 **[6]**. Given high levels of endemic CHC infection in Egypt, and that IR is a potentially modifiable factor, a better understanding of correlations of IR with HCC among Egyptian patients infected with HCV-4 is urgently needed.

PATIENTS AND METHODS

This study was conducted in Tropical Medicine Department, Zagazig University Hospitals in the period between June 2014 and June 2015. Three hundred and fifty two patients were included. The patients were divided into two groups: Group I which included 176 patients; 120 males and 56 females with post-hepatitis C virus cirrhosis, Child grade A and B without HCC and Group II which included 176 patients; 136 males and 40 females with post-hepatitis C virus cirrhosis, Child grade A and B with HCC.

Patients included in this study had chronic hepatitis C virus infection evident by positive serum markers, quantitative PCR for HCV-RNA and with post-hepatic cirrhosis diagnosed by pelvi-abdominal sonographic findings characteristic for liver cirrhosis without or with hepatocellular carcinoma diagnosed with special pattern in pelvi-abdominal triphasic CT.

Child C liver cirrhotic patients, liver cirrhotic patients of non HCV etiology, diabetic, hypertensive and dyslipidemic patients were excluded from the study. Patients with BMI > 30 kg/m^2 and those receiving any drug that may increase or decrease their insulin sensitivity were also excluded from the study.

All study patients were subjected to full history taking, thorough clinical examination, body mass index calculation, routine laboratory investigations (CBC, LFT, KFT and coagulation profile), other laboratory investigations (fasting and two hours postprandial blood glucose levels, lipid profile, viral markers, HCV RNA PCR, autoimmune markers and AFP).

Fasting serum insulin level was estimated for all patients by using active Insulin Enzyme-Linked Immunosorbent Assay (ELISA) DSL-10-1600 Diagnostic Systems laboratories, Inc. Corporate Headquarters, 445 Medical Center Blvd. Webster, Texas 77598-4217 USA. Homeostasis model assessment (HOMA) test to assess insulin sensitivity of the patients was calculated from the following equation:

HOMA-IR = fasting insulin (µU/mL) x fasting glucose (mmol/L) / 22.5
Or
HOMA-IR = fasting insulin (µU/mL) x fasting glucose (mg/dL) / 405

Mean HOMA -IR is 2.6 ± 0.14 in non-diabetic population and cut off value of HOMA-IR= 3 **[11]**. The reference values for HOMA-IR are Normal: HOMA-IR<2.60, Borderline: HOMA-IR 2.60-2.99 and Insulin resistant: HOMA-IR≥3 **[12]**.

Pelvi-abdominal ultrasonography was done for all patients. Findings suggesting cirrhosis and portal hypertension include increased echogenicity, coarse echotexture, hypertrophy of the caudate lobe and lateral segments of left lobe with concomitant atrophy of the posterior segments of the right lobe, dilated portal vein > 13 mm, splenomegaly and ascites **[13]**. Findings suggesting hepatocellular carcinoma include a small hypo-echoic lesion with poorly defined margins and heterogenous internal echoes **[14]**.

Triphasic CT confirmed diagnosis of HCC in group II patients. The specific imaging pattern is defined by intense contrast uptake during the arterial phase followed by contrast washout during venous or delayed phases. A pseudocapsule, a mosaic pattern, intralesional calcifications and intralesional fat may also be additional criteria. HCC can have three distinct patterns of growth: a single large tumor, multiple tumors and poorly defined tumor with an infiltrative growth pattern **[14]**.

Statistical Analysis

All data were analyzed using SPSS 15.0 for windows (SPSS Inc. , Chicago, IL, USA) & MedCalc 13 for windows (MedCalc Software bvba). Continuous data were expressed as the mean ± SD & median (range) and categorical data were expressed as a number (percentage). Continuous data were checked for normality by using Kolmogorov-Smirnov test. Student t-test was used to compare two groups of normally distributed continuous data, Mann-Whitney U

(MW) test for two groups of non-normally distributed continuous data. Categorical data were compared using the Chi-square (χ^2) test.

The Spearman's rank correlation coefficient was calculated to assess the correlations between HOMA-IR & various study parameters. A multivariate linear regression analysis was performed to test the effects of all study variables on HOMA-IR. A stepwise logistic regression analysis (SE: Standard Error, OR: Odd Ratio) was performed to assess the influence of various predictors of HCC development in multivariate model.

ROC curve analysis was determined to assess role of HOMA-IR in prediction of HCC development.

For two tailed tests P <0.025 was considered statistically significant (S), P<0.001 was considered highly statistically significant (HS), and P>0.025 was considered statistically non significant (NS). For one tailed tests P<0.05 was considered statistically significant (S), P < 0.005 was considered highly statistically significant (HS), and P>0.05 was considered statistically non significant (NS).

RESULTS

There were highly significant statistical increase in group II compared with group I as regard: fasting insulin (13.52 ± 7.32 vs 10.9 ± 6.107), HOMA-IR score (3.51 ± 1.43 vs 2.72 ± 1.14) and degree of IR (a total of 63.6% of patients of group II vs 44.3% of patients of group I with moderate and severe degrees of IR). It also shows no significant statistical difference concerning HbA1c between both groups (Table 1 & Fig. 1).

Also, HOMA-IR was a poor screening test for HCC (AUC=0.658, CI=0.606 – 0.708, p<0.001), using HOMA-IR as screening test at cutoff value = 1.07 & do test for 100 patient without HCC, we can accurately exclude only 2% of them that they haven't HCC & falsely diagnose 98% that they have HCC (Table 2 & Fig. 2).

In logistic regression analysis for predictors for HCC, it was found the relevant independent variables that could be used to predict occurring of HCC were HOMA-IR score and Child score, a model that correctly classified 65.3% of cases of HCC (Table 3).

Table (1): Comparison of studied groups as regards fasting serum insulin, frequency of degrees of fasting insulin level, HOMA-IR, HbA1c and frequency of different degrees of insulin resistance

		Group I N=176		Group II N=176		Test value (MW)	P value	Sign.
		Mean ± SD		Mean ± SD				
Fasting insulin (µU/mL)		10.9 ± 6.107		13.52 ± 7.32		11244	<0.001*	HS
		No	**%**	**No**	**%**	**X^2**	**P value**	**Sign.**
Fasting insulin	**Normal**	82	46.59%	66	37.5%	30.377	<0.00001*	HS
	Borderline	50	28.4%	20	11.36			
	High	44	25%	90	51.13%			
		Mean ± SD		**Mean ± SD**		**(MW)**	**P value**	**Sign.**
HOMA-IR		2.72 ± 1.14		3.51 ± 1.43		10592	<0.001*	HS
HbA1c		5.57 ± 0.53		5.74 ± 0.81		14302	0.213	NS
		No	**%**	**No**	**%**	**X^2**	**P value**	**Sign.**
IR	**Normal**	96	54.5%	64	36.4%	19.543	0.0001*	HS
	borderline	2	1.1 %	0	0%			
	Moderate	72	40.9%	90	51.1%			
	Severe	6	3.4%	22	12.5%			

Table (2): ROC Curve Analysis of HOMA-IR as screening test for HCC

Cut-off values	Sens. % (95% CI)	Spec. % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	AUC* (95% CI)
> 1.07	100 % (97.9-100)	2.27 % (0.6-5.7)	50.6 % (45.2-55.9)	100 % (39.8-100)	0.658‡ (0.606-0.708)

* Accuracy of HOMA-IR = poor
‡ p < 0.0001 **(HS)**

Table (3): Logistic regression analysis for predictors for HCC

	Regression Coefficient	SE	OR	95% CI	p	Sig.
HOMA-IR	+ 0.517	0.092	1.678	1.401 – 2.009	0.0001	**HS**
Child score	- 0.330	0.124	0.719	0.564 – 0.917	0.008	**S**
HE	+ 0.234	0.289	1.264	0.717 – 2.227	0.418	**NS**
Ascites	+ 0.221	0.245	1.247	0.771 – 2.016	0.368	**NS**
Constant	+ 0.485	0.761	---	---	0.524	**NS**

p = 0.004 **(S)**
Variable not included in the model were; age, Hb, INR, bilirubin, ALT, Alpha fetoprotein, fasting insulin level, liver span
The model correctly classified 65.3 % of cases
Logistic Regression Equation according this model
HCC (>0) = 0.517 × HOMA-IR - 0.330 × Child score

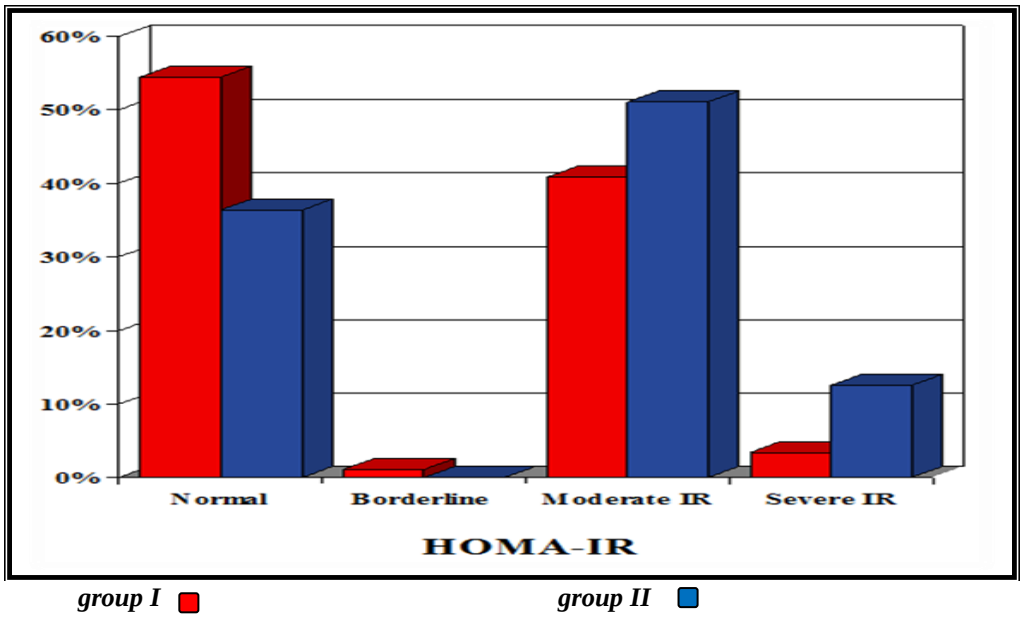

Fig. (1): Bar chart shows comparison between both groups regarding degree of IR

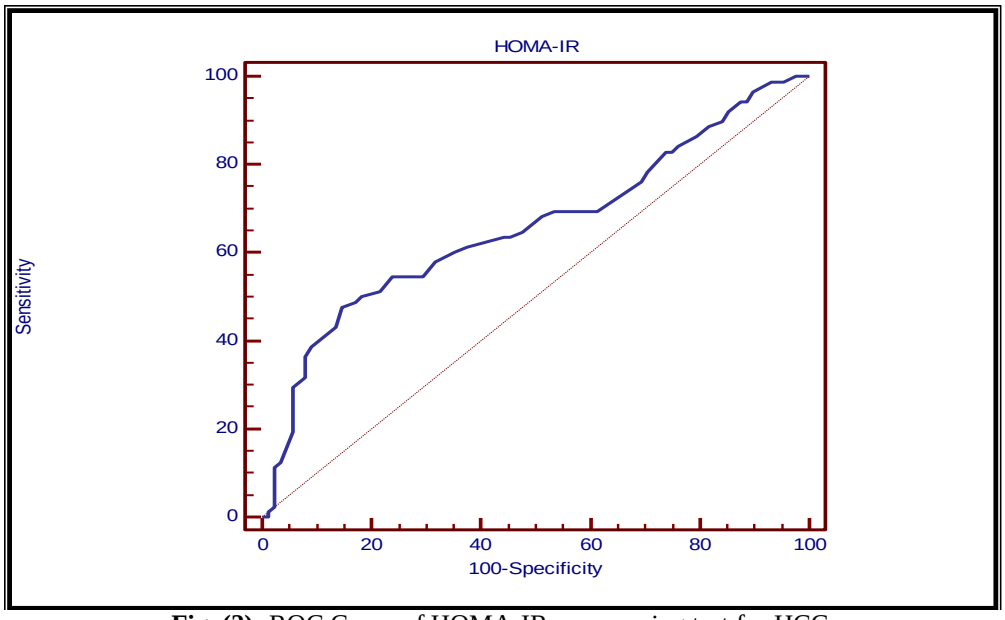

Fig. (2): ROC Curve of HOMA-IR as screening test for HCC

DISCUSSION

Hepatitis C virus (HCV) infection is one of the most serious global health problems and major causes of chronic liver disease. According to recent WHO estimates, the incidence of HCV infection is increasing with over 185 million people infected worldwide **[15]**. Moreover, approximately 370000 HCV-infected individuals die of liver-related causes each year [16].

Insulin resistance is frequently seen in patients with hepatitis C virus (HCV) infection **[17]**. Lack of exercise and overeating are major causes of insulin resistance in the general population. 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 **[18]**. Thus, 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 **[19]**.

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 **[20]**. The development of hepatic complications, including hepatocellular carcinoma (HCC) on the other hand, is known to be associated with diabetes mellitus **[21]**.

In this study, we aimed at determining the frequency of insulin resistance in patients with chronic HCV and in patients with HCV-associated HCC in Zagazig University Hospitals, to discuss the severity of insulin resistance in HCV-associated HCC patients and in chronic HCV patients with cirrhosis, in order to be able to recommend further research on possible management modalities for later patients, with the hope of hindering the progressive pathway of HCV cirrhosis to eventual hepatocellular carcinoma.

In our study, there was no significant statistical difference between group I and group II as regard Child-Turcotte-Pugh grade, however, grade B was more prevalent in both groups, being 62.5% of patients in group I and, 61.4% of patients in group II. We excluded patients with Child C grade from the study as they weren't expected to benefit from the results.

Regarding fasting plasma insulin levels in both groups of our study , a highly significant statistical increase was found in group II (mean 13.52±7.32) compared with group I (mean 10.9±6.1), a finding that is strongly comparable with HOMA-IR being highly significantly increased in group II(mean 3.51±1.43) compared to group I (mean 2.72±1.14) , a finding that is in accordance with **Hung et al. [22]** who concluded that patients with hepatocellular carcinoma had higher HOMA-IR values than those with chronic hepatitis and advanced fibrosis.

Irshad et al. [23] found that HOMA-IR > 3 is present in 33.33% of patients with cirrhosis due to HCV infection while it is 45.5% in patients with HCV associated hepatocellular carcinoma. **Donadon et al. [24]** also concluded that the mean values of fasting insulin and HOMA-IR increase progressively in liver cirrhosis and hepatocellular carcinoma.

Degree of Insulin resistance (HOMA-IR ≥3) was higher in patients of group II, 51.1% moderate (HOMA-IR = 3-5) and 12.5% severe IR (HOMA-IR >5), compared to patients of group I, 40.9 % moderate and 3.4% severe IR.

Moreover, we found highly significant statistical increase in fasting plasma insulin level in patients of group II in comparison to patients of group I. Our results showed that in group II; 51.1% of patients had high fasting insulin level (≥12µU/mL) compared to only 25% of patients of group I.

In a cross sectional study of hospitalized patients with chronic HCV liver cirrhosis and HCC, **Donadon et al.[24]** found that IR, and the consequent hyperinsulinemia, are 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, i.e ; both the degree of insulin resistance and the fasting insulin level increase as the liver disease progresses.

In **N'kontchou et al. [25]** studied the risk factors for HCC in patients with viral and alcoholic cirrhosis and found a positive linear relationship between BMI and development of HCC.

In our study, we tried to determine the efficiency of HOMA-IR score as a screening test for HCC, using receiver operating characteristic (ROC) curve analysis. However, we concluded that HOMA-IR was a poor screening test for HCC i.e; although it has 98 up to 100 % sensitivity, it only has a specificity between 0.6 and 5.7 % with a mean of 2.27%, which means that with using HOMA-IR at cut-off value >1.07, for each 100 patients without HCC, we could accurately exclude 2% of them being free of HCC and falsely diagnose 98% of them as HCC patients.

In logistic regression analysis for predictors for HCC, we studied the relationship between different independent variables and a dependent variable, which is occurring of HCC, and concluded that, after controlling age, hemoglobin level, INR, bilirubin, ALT, alpha-fetoprotein, fasting insulin level and liver span, the relevant independent variables that could be used to predict occurring of HCC were HOMA-IR score and Child score, a model that correctly classified 65.3% of cases of HCC.

So we concluded that frequency of insulin resistance was higher in patients with HCV-associated hepatocellular carcinoma (63.6%) than in patient with mere chronic HCV-associated cirrhosis (44.3%). Patients with HCV-associated hepatocellular carcinoma had higher fasting insulin levels, higher HOMA-IR scores and higher degrees of insulin resistance than patients with chronic HCV-associated cirrhosis, i.e. percentage of patients with severe degree of insulin resistance in HCC group was about 4 times the percentage in non-HCC group. HOMA-IR is a poor screening test for detection of hepatocellular carcinoma.

Conflict of interest: No

REFERENCES

[1] **Marcellin P (1999):** " Hepatitis C : The clinical spectrum of the disease " J Hepatol; 31 (Suppl.1):9-16.

[2] **Lavanchy D (2011):** " Evolving epidemiology of hepatitis C virus" Clin Microbiol Infect.;17(2):107-15.

[3] **Center for Disease Control and Prevention, CDC Health Information for International Travel**.(**2012)**: New York: Oxford University Press.available At: http://wwwnc.cdc.gov/ travel/ yellowbook/ 2012/ chapter-3-infections-disease-related-to-travel/hepatitis-c.htm.

[4] **Abdel-Hamid M, El-Ghawalby N, Mostafa M, et al., (2007):** Epidemiology of hepatocellular carcinoma in lower Egypt, Mansoura Gastroenterology Center. Hepatogastroenterology; 54: 157 – 62**.**

[5] **Persico M , Persico E, Suozzo R et al.,(2000):** " Natural history of hepatitis C virus carriers with persistently normal aminotransferases levels " Gastroenterology;118(4):740-4.

[6] **Sheik M, Choi J, Qadri I et al.,(2008):** " Hepatitis C virus infection : molecular pathways to metabolic syndrome " Hepatology;47:2127-2133.

[7] **El-Serag H (2001):** " Epidemiology of hepatocellular carcinoma " Clin Liver Dis;5(1):87-107.

[8] **Velazquez R , Rodriguez M, Navascues C et al.,(2003):** " Prospective analysis of risk factors for HCC in patients with liver cirrhosis" Hepatology;37(3):520-527.

[9] **El-Zayadi A, Abaza H, Shawky S et al.,(2001):**"Prevalence and epidemiological features of HCC in Egypt: a single centre experience" Hepatology Res;19:170-179.

[10] **Calle E,Rodriguez C and Walker-Thurmond K (2003): "**Overweight, obesity and mortality from cancer in a prospectively studied cohort ofU.S adults ".N Eng. J Med ;348:1625-38**.**

[11] **Ikeda Y, Suehiro T, Nakamura T et al.,(2001):** " Validity and reproductivity of HOMA-IR" J Clin Endocrinol Metab;86:713-718.

[12] **Hayes M, Miller C , Ulbrecht J et al.,(2007):** " A carbohydrate-restricted diet alters gut peptides and adiposity signals in men and women with metabolic syndrome" J Nutr;137(8)1944-50.

[13] **Chung-Chieng W (2008):** " Ultrasonographic evaluation of portal hypertension and liver cirrhosis " Journal of Medicine Ultrasound ;16(3):188-193.

[14] **Colli A, Fraquelli M, Casazza G et al.,(2006):** " Accuracy of Ultrasonography, spiral CT, magnetic resonance and alphafeto protein in diagnosing hepatocellular carcinoma : A systematic review " Am J Gastroenterol ;101(3):513-23.

[15] **Mohamed Hanafiah K, Groger J and Flaxman A (2013):** " Global epidemiology of hepatitis C virus infection : new estimates of age-specific antibody to seroprevalence" Hepatology;57:1333-1342.

[16] **Perz J, Armstrong G, Farrington L et al.,(2006):** " The contributions of HBV and HCV infections to cirrhosis and 1ry liver cancer worldwide" J Hepatol;45:529-538.

[17] **Caronia S, Taylor K, Pagliaro L et al.,(1999):** " Further evidence for an association between non-insulin dependent diabetes mellitus and chronic hepatitis C virus infection" Hepatology;30:1059-63.

[18] **Eguchi Y,Mizuta T, Ishibashi E et al.,(2009):**" Hepatitis C virus infection enhances insulin resistance induced by visceral fat accumulation" Liver Int;29:213-220.

[19] **Kawaguchi T, Nagao Y, Tanaka K et al.,(2005):** "Causal relationship between hepatitis C virus core and the development of type 2 diabetes mellitus in a hepatitis C virus hyperendemic area : a pilot study" Int J Mol Med;16:109-14.

[20] **Kawaguchi T, Yoshida T, Harada M et al.,(2004): "** Hepatitis C virus downregulates insulin receptor substrates 1 and 2 through upregulation of suppressor of cytokine signaling 3" Am J Pathol;165:1499-1508.

[21] **Davila J, Morgan R, Shaib Y et al.,(2005):** " Diabetes increases the risk of HCC in the United States : a population based case control study" Gut;54:533-539.

[22] **Hung C, Jing-Houng W, Tseng-Hui H et al.,(2010):** "Insulin resistance is associated with HCC in chronic hepatitis C infection" World J Gastroenterol;16(18):2265-2271.

[23] **Irshad M, Iqbal A, Ansari M et al.,(2013):**" Relation of insulin resistance with viral etiology and blood level of cytokines in patients with liver disease" Global Advanced Research Journal of Medical science;2(3):075-083.

[24] **Donadon V, Balbi M, Mas M et al., (2010):** "Metformin and reduced risk of HCC in diabetic patients with chronic liver disease" Liver Int; 30:750-758.

[25] **N'kontchou G, Paries J, Htar M et al.,(2006):** "Risk factors for HCC in patients with alcoholic or viral hepatitis C cirrhosis " Clin Gastroenterol Hepatol;4:1062-1068.