ISSN NO. 2320-5407

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

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Study of Interleukin-8 Gene Polymorphisms in Egyptian Hepatocellular Carcinoma Patients and Association with Insulin Resistance State

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Manuscript Info

Manuscript History:

Received: 11 November 2014
Final Accepted: 22 December 2014
Published Online: January 2015

Key words:
Gene polymorphism, Hepatocellular carcinoma, Insulin resistance, Interleukin-8

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Abstract

Hepatocellular carcinoma is the six cancer worldwide and the first in Egypt . Interleukin-8 is an angiogenic chemokine with important roles in development and progression of many human malignancies including HCC. This study was designed to investigate the association of IL-8(- 251A/T and +781 C/T) Single-Nucleotide-Polymorphisms with susceptibility of HCC in patients with chronic hepatitis C and to correlate it with insulin resistance. 112 HCV related HCC patients and 105 control subjects were analyzed for serum IL-8, fasting insulin level using (ELISA- technique) and IL-8(-251A/T and +781 C/T) SNPs using PCR-RFLP genotyping analysis, then HCV related HCC patients were subclassified into 79 patients with IR and 33 patients without IR . Results showed that individuals with IL-8 +781 T/T homozygote have lower risk of HCC development (with OR=0.26) while others with C allele have a higher risk (with OR=2.01) and T allele of IL-8 (+781 C/T) in HCC subjects with and without IR show significant for (AOR) =0.59 while no significant for (OR=0.65). It could be concluded that IL-8(-251 and +781) SNPs can considered to be a candidate gene for the liability of HCC also, could predict the prognosis of the progression of chronic HCV infection to HCC or not. Moreover, IR and steatosis could be a complicating event of chronic HCV and could be considered as a possible predisposing factor for HCC in such patients inspite of there is found correlation with the studied SNPs in IL-8 gene which need further extended studied to validate these results.

INTRODUCTION

Hepatocellular carcinoma is the most common primary malignant liver tumor and one of the most common causes of cancer mortality in the world. It usually arises on top of cirrhotic liver. It has high incidence rate above 40 years with male to female ratio about 8:1 with more than 90% mortality rate. It is estimated that HCC is responsible for more than 600,000 deaths annually worldwide **[1].**

The burden of HCC has been increasing in Egypt with doubling in the incidence rate in the past 10 years **[2].** Many risk factors predispose to HCC, these risk factors may present individually or collectively depending on the environmental situations such as hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholism, aflatoxin (AF), schistosomiasis and some hereditary diseases as haemochromatosis and haemophilia **[3].**

Obesity and the metabolic syndrome (MS) are growing epidemics associated with an increased risk for many types of cancer. In the liver, inflammatory and angiogenic changes due to insulin resistance and fatty liver disease are associated with an increased incidence of liver cancer. Regardless of underlying liver disease, cirrhosis remains the most important risk factor for hepatocellular carcinoma (HCC) although rare cases of HCC arising without cirrhosis

raise the possibility of a direct carcinogenesis secondary to nonalcoholic fatty liver disease (NAFLD). Moreover, MS and its different features may also increase the risk of HCC in the setting of chronic liver diseases of other causes such as viral hepatitis or alcohol abuse. Taking into account all these data, it is necessary to better determine the risk of developing HCC in patients with MS to improve the screening guidelines and develop prophylactic treatments in this setting **[4].**

Numerous population studies have revealed strong links between obesity and the development of liver cancer. Obesity can alter hepatic pathology, metabolism and promote inflammation, leading to nonalcoholic fatty liver disease (NAFLD) and the progression to the more severe form, non-alcoholic steatohepatitis (NASH). NASH is characterized by prominent steatosis and inflammation, and can lead to HCC **[5].**

Non-alcoholic steatohepatitis (NASH) is the liver manifestation of metabolic syndrome, which constellates obesity, insulin resistance and dyslipidemia. It is correlated significantly in many studies with HCC. Recent evidence also indicates the significant role of genetic factors in contributing to the pathogenesis of NASH and induced hepatic malignancy **[6].**

Interleukin-8 (IL-8) is a multifunctional chemokine that affects human neutrophil functions, including chemotaxis, enzyme release, and expression of surface adhesion molecules **[7].** It has direct effects on tumor and vascular endothelial cell proliferation, angiogenesis, and tumor migration **[8]** of several tumor types, including prostate, breast, colon, lung, ovarian, and liver cancers **[9].**

The IL-8 gene, located on chromosome 4q13–q21 in humans, is composed of four exons, three introns, and a proximal promoter region. A common SNP at position -251 of the IL-8 promoter region was identified recently and consequent evidences demonstrate that IL-8 - 251A/T polymorphism associates with IL-8 production or protein expression both in vivo and in vitro **[10].** SNP in relation to HCC has yet to be evaluated in Egypt. In addition to IL-8 -251 A/T, previous studies report the association between other IL-8 genetic SNPs, three IL-8 SNPs including +781 C/T, +1633 C/T, and +2767 A/T have been studied in Taiwanese population **[11].**

The aim of the current study was to investigate the association of IL-8 - 251A/T and +781 C/T SNPs with HCV related HCC and to find a possible relation to insulin resistance state in sample of Egyptian patients attending Mansoura University Hospitals.

1. Materials and Methods

2.1 Materials

This hospital-based case-control study included 112 HCC patients recruited prospectively from out and inpatient clinics of Tropical Medicine Department, Mansoura University during the period from March 2011 to March 2014. The study was approved by the ethical committee of the faculty and informed consents were obtained form all subjects.

Subjects in this study were classified into: 112 patients with HCC on top of chronic HCV infection with no steatosis (HCV related HCC group) and 105 healthy volunteers as a control group and we make certain they were healthy by examining them for hepatitis C virus& HBsAg which were Negative by ELISA ,also All controls were subjected to Abdominal ultrasound investigation which show they are healthy. Subjects of HCV related HCC group were sub-classified into HCC with IR (79 patients) and HCC with no IR (33 patients).

HCC was diagnosed according to the diagnostic guidelines of the European Association for the Study of the Liver **[12].** Exclusion criteria included are: Patients with liver diseases other than HCC such as (Primary Billary Cirrhosis, Primary Sclerosing Cholongitis, Toxic hepatitis, Wilson disease, Tyrosinemia), also Patients with HCC but with no cirrhosis or with AFP level less than 200 ng/dl, patients with liver metastasis as well as HIV infected patients. also usage of Nexavar was excluded.

2.2 Methods

Samples collection: 2 ml venous blood samples were delivered to sterile collection tubes containing K_2EDTA (Stored as EDTA anticoagulated blood Samples at -70^{o}C for DNA extraction and genotyping of Interleukin -8 gene using the specific restriction enzyme (RFLP). Another 5 ml blood samples were delivered to Vacuum blood collection tubes and allowed to clot for 15 minutes and centrifuged at 7000 rpm for 10 minutes for serum separation then serum collectrd in other sterile tubes and stored at - 70^{o}C until used to determine serum α fetoprotein, IL-8 serum level and Lipid profile. In addition, fasting blood glucose and insulin levels were determined for estimation of HOMA-IR.

DNA Extraction: Genomic DNA was extracted from EDTA-anticoagulated peripheral blood leucocytes using G-spin™ Total DNA Extraction Kit supplied by intron biotechnology, IBT-QMS-GT1704(R01-2012-01.The average DNA concentration was 0.127±0.005µg/µl determined by measuring the absorbance at 260 nm and 280 nm (Jenway, Genova Model, UK). All samples had a 260/280 nm absorbance ratio between 1.6 and 1.79. The integrity of the DNA was checked by electrophoresis on 0.8 % agarose gel stained with ethidium bromide using Gel electrophoresis apparatus.

Genotyping of IL-8 gene - 251A/T and +781 C/T Single Nucleotide Polymorphisms (Chien et al., 2011) :

Polymerase Chain Reaction (PCR): The primers sequences used for DNA amplification are : F 5'-TCATCCATGATCTTGTTCTAA-3',R 5'-GGAAAACGCTGTAGGTCAGA-3'for IL-8 - 251A/T SNP and F 5;-CTCTAACTCTTTATATAGGAATT-3,R 5'-GATTGATTTTATCAACAGGCA-3' for IL-8 +781 C/T SNP.

. PCR was carried out in 50 µL final reaction volume using 2X PCR Master mix Solution (*i*-Taq ™) INTRON BIOTECHNOLOGY (cat.No.25028).The following mixture was prepared for each sample: 25 µl PCR Master mix solution (2×), 1µl (20 pmole) of forward primer, 1µl (20 pmole) of reverse primer, 2µl (200ng) of genomic DNA and 21 µl of double distilled deionizer water. Amplification was performed in a Thermal Cycler (TECHEN TC-312, Model FTC3102D, Barloworld Scientific Ltd. Stone, Stafford Shire St., 150 SA, UK) using the following program: initial 5 minutes denaturation at 94°C followed by 35 cycles of denaturation at 94°C for 1 minute, annealing was done for 30 seconds at 58°C for IL-8 - 251A/T SNP and 52°C for IL-8 +781 C/T SNP , extension at 72°C for 2 minutes and a final extension for 20 minutes at 72°C.

Amplified samples were digested with the specific restriction enzyme; MfeI (MfeI-HF® New England Biolabs inc.-Cat. No. R3589S- 20,000 units/ml) and EcoRI (EcoRI- New England Biolabs inc.-Cat.No. R0101S- 20,000 units/ml) for IL-8 (- 251A/T SNP and +781 C/T SNPs) respectively according to the manufacturer's instructions.1µl (2 unit) of each restriction enzyme, 10 µl (1 µg)of PCR product in a 50 µl, 5 µl (1X) of 10X NE Buffer and 34 µl distilled water to obtain 50 µl total Reaction Volume ,incubated for one hour at 37°C.

This enzyme the digestion products were electrophoresed on a 3.0% agarose gel for 60 min, stained with ethidium bromide using Gel electrophoresis apparatus, visualized via Light UV Transilluminator (Model TUV-20, OWI Scientific, Inc. 800 242-5560, France) and photographed. IL-8 – 251 A/T- MfeI restriction enzyme digestion product are: T/T: 524 bp, A/A: 449 bp, 75 bp ,while ,T/A: 524 bp, 449 bp, 75 bp. IL-8 +781 C/T EcoRI restriction enzyme digestion product are: T/T: 203 bp , C/C: 184 bp, 19 bp and T/C : 203 bp,184 bp, 19 bp.

Estimation of serumHuman Interleukin 8 levels

Quantitative determination of serum Human Interleukin-8 levels was performed by Human interleukin-8 ELISA Kits (Invitrogen- Catalog # KHC0081: 96 Tests - 542 Flynn Road, Camarillo, CA 93012). This assay employs the quantitative sandwich ELISA technique. It was performed according to the manufacturer's instructions. The absorbance of each sample was read on plate ELISA reader (Tecan, Sunrise, Austria) at 450 nm.

Homeostasis Model Assessment of Insulin Resistance (HOMA-IR):

In each sample, the degree of insulin resistance was estimated by the homeostasis model assessment (HOMA-IR) as described by **[13].** HOMA-IR was calculated by taking into account fasting insulin and blood glucose levels according to the equation (HOMA-IR) = fasting insulin (µU/ml) × fasting blood glucose mg/dl) ×0.0551/ 22.5. Serum insulin level was estimated using ELISA Kit **[14].** The kit was provided from Diagnostic Systems laboratories. Inc. Corporate Headquarters, 445 Medical Center, Blvd. Webster, Texas 77598-4217 USA. Fasting blood glucose level was done according to method of **[15]** and The kits were provided from Elitech diagnostics, Zone Industrielle 61500, Sees France.

Estimation of serum α faetoprotein (AFP)

Serum level of α faetoprotein (AFP) was estimated using alpha Fetoprotein Human ELISA Kit (catalog number ab108838, Abcam, alpha Fetoprotein Human). HCV antibody and HbsAg were estimated by An enzyme immunoassay (ELISA) for the qualitative detection of IgG antibodies to Hepatitis C Virus (HCV) **[16]** and HBsAg **[17]** in human serum using RUO kits (catalog number; 6307125& 6307105 respectively)- LINEAR CHEMICALS S.L. Joaquim Costa 18 2ª planta. 08390 Montgat, Barcelona, SPAIN. HCV infection was confirmed by the Cobas TaqMan HCV test real-time RT quantifiable PCR for HCV.

Estimation of serum Lipid profile

Lipid profile estimation were performed colorimetrically using the commercially available kits. Serum triglycerides level was estimated according to the method of **[18]** and Serum total cholesterol was estimated according to the method of **[19].**The kits were provided from Elitech diagnostics, Zone Industrielle 61500, Sees France. While; Serum high-density lipoprotein (**HDL**) cholesterol was estimated according to the method of **[20]**. The kits for HDL cholesterol precipitation were provided from Stanbio Laboratory, 1261 North Main Street, Boerne, Texas.

Serum **LDL**- Cholesterol was estimated using polyvinyl sulphate method **[21].** The precipitant solution was supplied from Quimica Clinical Aplicada S. A. Amposta / Spain.

2. Statistical Analysis

The data were expressed as mean, standard deviation (±SD) for the studied groups. Statistical analysis was performed using statistical package for social science (SPSS) program version 13 (SPSS Inc., Chicago, IL, USA). The studied groups were compared by Chi square (X^2) test to evaluate Hardy-Weinberg equilibrium in allele and genotype frequencies in the study group. The association of IL-8 gene polymorphisms with hepatocellular carcinoma with steatosis risk was estimated using odds ratios (OR) and 95% confidence intervals (95% CI) for the comparison of genotype and allele contrast. The difference was considered statistically significant when *P* value was less than 0.05.

3. Results

As shown in table (I); There is significant differences for the measured parameters (Body Mass Index (BMI), homeostasis model assessment (HOMA-IR), Total Cholesterol, Triglycerides (TG), High-Density Lipoprotein Cholesterol (HDLC), Low-Density Lipoprotein Cholesterol (LDLC), α faetoprotein (AFP) and IL-8) With (p value<0.001) when compared HCV related HCC group to the control group.

In table (II); The IL-8(-251 A/T genotype) distribution and allele frequencies showed NO significant differences between control group and HCV related HCC patients group. While; IL-8 (+781 C/T genotype) distribution show significant differences in genotype frequency (TT, (CT+TT) and allele frequencies), while no significant difference in genotype frequency CT in comparing control group and HCV related HCC patients group. Also in IL-8(+781 C/T) polymorphism subjects with IL-8 +781 T/T homozygote have lower risk for HCC development than other genotype frequencies with (OR=0.26). Subjects with C allele have higher risk of HCC development than all genotypes and allele frequency have been studied with (OR=2.01).While subjects with T allele have the lowest risk of HCC with (OR=0.05). Also, we found that A/C haplotype of IL-8 -251/+781 associated with higher risk with susceptibility to HCC occurrence than others with (OR=2.17).

In table (III); There is significant differences in all of the studied biochemical parameters when compared between HCC with IR patients group and HCC without IR patients group.

Table(IV) ;The IL-8(-251 A/T & +781 C/T genotype) distribution and allele frequencies showed NO significant differences between HCC with IR Patients group and HCC without IR Patients group. Except T allele of (+781 C/T genotype) have statistical significant for Adjusted Odd Ratios (AOR) with (p value=0.01).

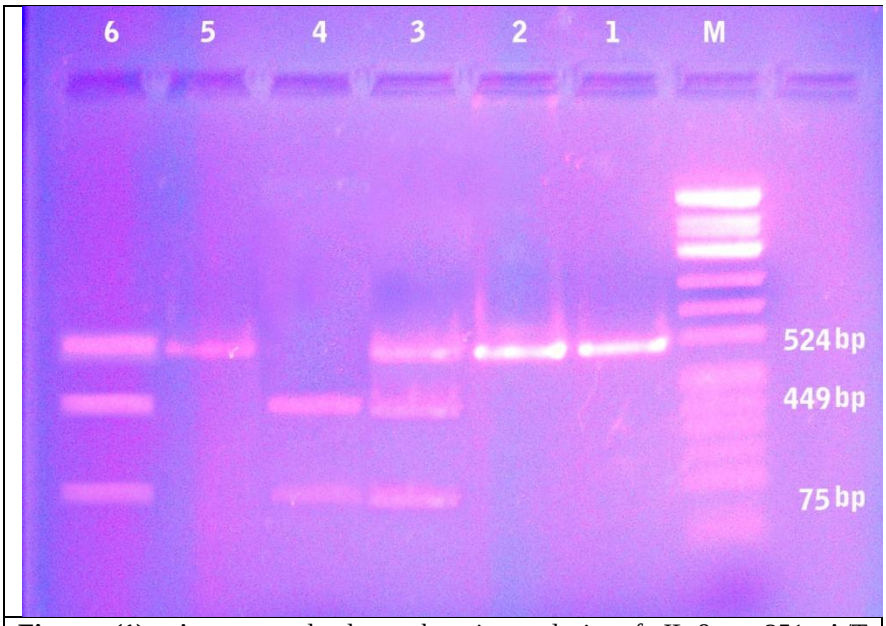

Figure (1): Agarose gel electrophoretic analysis of IL-8 – 251 A/T Polymorphism after MfeI digestion analysis. Lane (M) represents the molecular marker .Lanes (1&2&5) represent T/T genotype showing one band at 524 bp. Lane (3&6) represents the T/A genotype showing three bands at 524,449& 75 bp. Lane (4) represents the A/A genotype showing two bands 449 & 75 bp.

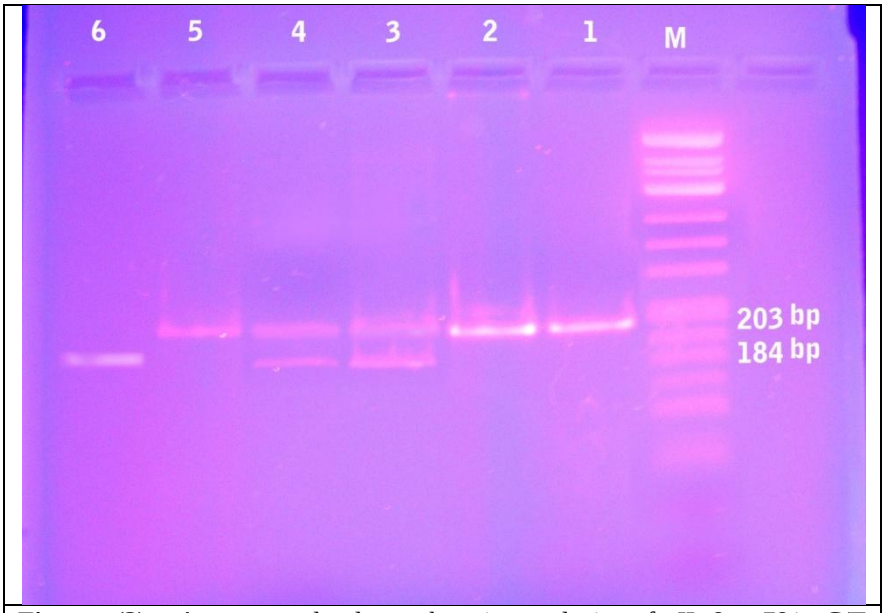

Figure (2): Agarose gel electrophoretic analysis of IL-8 +781 C/T Polymorphism after EcoRI digestion analysis. Lane (M) represents the molecular marker .Lanes (1,2&5) represent T/T genotype showing one band at 203 bp. Lane (3&4) represents the T/C genotype showing three bands at 203,184& 19 bp. Lane (6) represents the C/C genotype showing two bands 184 & 19 bp. Band at 19 bp is not clearly seen due to running faster and getting out of the gel.

Table I: The measured Parameters in the studied groups

	Control	HCV related HCC	P value
Number	105	112	
Age (year)	45.8± 7.2	47.3±5.1	0.080
Sex: male No (no%) Female No (no%)	56 (53.3%) 49 (46.7%)	67 (59.8%) 43 (40.2%)	0.320
BMI (kg/m^2)	25.1±4.3	33.5±6.6	< 0.001
HOMA-IR	2.13±0.91	6.11±1.14	< 0.001
Total cholesterol (mg/dL)	135.1±25.2	193.5±35.2	< 0.001
TG (mg/dL)	78.6±16.3	113.32±25.2	< 0.001
HDL-C (mg/dL)	58.8±9.5	39.4±8.2	< 0.001
LDL-C (mg/dL)	90.5±11.3	119.8±7.8	< 0.001
AFP (ng/mL)	8.9±4.3	1285.5±434.6	< 0.001
IL-8 (ng/L)	13.6±11.3	415.9±213.3	< 0.001

P value < 0.05 considered statistically significant

Table II: Genotype, Allelic frequency and Haplotyping of IL-8 gene polymorphism in Control and HCC groups.

	Control	HCC	OR	P	AOR	P
Number	105	112				
IL-8(-251 A/T genotype)						
TT	33(31.4 %)	29(25.9 %)	1			
AT	49(46.7%)	56(50%)	1.14 (0.65-2.02)	0.13	0.92(0.52-1.63)	0.36
AA	23(21.9%)	27(24.1%)	1.13(0.57-2.24)	0.83	0.76(0.4-1.45)	0.45
AT+AA	72(68.6%)	85(75.9%)	1.32(0.7-2.47)	0.45	1.8(0.92-3.66)	0.08
A allele	82(53.2%)	85(50.6%)	1.13(0.75-1.71)	0.62	1.3(0.86-2.0)	0.22
T allele	72(46.8%)	83(49.4)	0.92(0.61-1.38)	0.74	0.68(0.45-1.04)	0.08
IL-8(+781C/T genotype)						
CC	36(34.3%)	68(60.7%)	1			
CT	45(42.9%)	36(32.2%)	0.63(0.34-1.14)	0.140	0.65(0.36-1.17)	0.160
TT	24(23.9%)	8(7.1%)	0.26(0.1-0.65)	0.002	0.41(0.17-0.96)	0.040
CT+TT	69(65.7%)	44(39.3%)	0.34(0.19-0.61)	<0.001	0.42(0.23-0.75)	0.002
C allele	81(54%)	104(70.3%)	2.01(1.21-3.34)	0.006	1.93(1.16-3.21)	0.010
T allele	69(46%)	44(29.7%)	0.05(0.3-0.82)	0.006	0.52(0.31-0.86)	0.010
Haplotyping						
$A^{-251}.C^{+781}$			2.17(1.32-3.57)	0.001		
$A^{-251}.T^{+781}$			0.87(0.48-1.54)	0.620		
$T^{-251}.C^{+781}$			0.76(0.47-1.23)	0.290		

$T^{-251}.T^{+781}$			0.6(0.33-1.09)	0.100		

Table III: **The measured parameters for HCC with and without IR patients groups.**

	HCC with IR	HCC without IR	P value
Number	79	33	< 0.001
BMI (kg/m^2)	34.3±5.1	28.2±4.3	< 0.001
HOMA-IR	5.81±1.1	2.9±0.9	<0.001
Total cholesterol (mg/dL)	201.1±41.3	156.3±29.5	< 0.001
TG (mg/dL)	119.3±31.7	99.3±21.8	< 0.001
HDL-C (mg/dL)	37.8±11.3	43.1±7.8	0.020
LDL-C (mg/dL)	123.8±17.3	89.3±7.8	<0.020
IL-8 (ng/L)	397±176.3	269.3±18.7	< 0.001

Table IV: Genotype, Allelic frequency and Haplotyping of IL-8 gene polymorphism in HCC with and without IR.

	HCC with IR	HCC without IR	OR	P	AOR	P
Number	79	33				
IL-8(-251 A/T genotype)						
TT	19(24.1 %)	10(30.3 %)	1			
AT	37(46.9%)	12(36.4%)	1.54(0.62-3.87)	0.42	1.32(0.53-3.33)	0.66
AA	23(29.1%)	11(33.3%)	0.82(0.32-2.15)	0.83	0.78(0.29-2.11)	0.76
AT+AA	60(76%)	23(69.7%)	1.37(0.51-3.71)	0.65	1.2(0.45-3.2)	0.87
A allele	56(48.3%)	22(49.9%)	0.98(0.46-2.06)	0.89	0.94(0.45-1.99)	0.85
T allele	60(51.7%)	23(51.1)	1.12(0.53-2.36)	0.89	1.16(0.55-2.44)	0.81
IL-8(+781C/T genotype)						
CC	50(63.3%)	18(54.6%)	1			
CT	23(29.1%)	13(39.4%)	0.63(0.25-1.61)	0.41	0.49(0.2-1.24)	0.15
TT	6(7.6%)	2(6%)	1.27(0.21-9.71)	0.77	1.51(0.26-11.18)	0.62
CT+TT	29(36.7%)	15(45.4%)	0.69(0.28-1.72)	0.52	0.79(0.32-1.96)	0.57
C allele	73(76%)	31(67.4%)	1.54(0.66-3.57)	0.38	1.69(0.73-3.9)	0.25
T allele	23(24%)	15(32.6%)	0.65(0.28-1.52)	0.38	0.59(0.26-1.36)	0.01
Haplotyping						
$A^{-251}.C^{+781}$			1.95(0.85-4.54)	0.13		
$A^{-251}.T^{+781}$			0.59(0.22-1.58)	0.35		
$T^{-251}.C^{+781}$			0.66(0.24-1.23)	0.52		
$T^{-251}.T^{+781}$			2.38 (0.67-8.64)	0.22		

1. Discussion:

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related death worldwide. Primary hepatocellular carcinoma can be found most frequently (80-90%) in patients with liver cirrhosis **[22].**

Park SY *et al* [23] reported that Interleukin-8 (IL-8) has been suggested as a prognostic biomarker for human hepatocellular carcinoma (HCC), but its roles in HCC progression and drug resistance have not been studied. Interleukin-8 (IL8) polymorphisms have been implicated in several cancers, but their roles in the pathogenesis of hepatocellular carcinoma (HCC) are largely unknown **[24].**

Many scientists identified the cytokine IL-8 as leukocyte chemoattractant with principal role in initiation and amplification of acute inflammatory reactions and one of the CXC chemokine families. The gene coding for IL-8 is within the CXC chemokine locus on chromosome 4 and consist of 4 exons **[23]**, in this study we examine only 2 sites -251 A/T and +781 C/T.

This case –control study identified subjects with IL-8 +781 T/T homozygote have lower risk of HCC development while subject with c allele have a higher risk ,while in IL-8 -251 A/T polymorphism subject with T allele have a statistical near significant higher risk of HCC development ,also we found that A/C haplotype of IL-8 -251/+781

associated with 2.17 fold of higher risk with susceptibility to HCC occurrence than other haplotypes A/T,T/C and T/T , haplotypes studies are more stronger as they test more than one gene .

Data of our study indicate that carriers of the IL-8 +781T/T homozygote may have decreased risk of HCC as compared to control subject (p = 0.04) in accordance to **Ren *et al.* [9]** who find decreased risk of HCC in these subjects ;previous studies of **Song *et al*. [26]** found significant association of IL-8 +781 T allele with susceptibility to cachexia in gastric cancer .

HCV has been identified as a cause of metabolic syndrome that includes dyslipidemia, diabetes and insulin resistance (IR) **[27].**In chronic HCV infection, IR can favor fibrosis progression directly and act indirectly by inducing steatosis in a genotype-4 dependent manner **[28].**

Veldt BJ *et al* [29] reported that 30 to 70% of HCC patients display some evidence of IR. The results of their studies have suggested the occurrence of IR at early stage(s) of chronic HCV infection irrespective of the severity of liver disease and thus the possible role of IR as a metabolic factor that increases risk of HCC development. **Douglas and George [30]** explained this by direct effects of HCV in modulating insulin signaling and molecular pathways involved in IR development in hepatocytes. In turn, hyperinsulinemia that results from IR stimulates growth of HCC and inhibits apoptosis in diabetic patients.

Chen CL *et al* [31] suggested a strong synergistic effect of metabolic factors and viral hepatitis in HCC development in HCV-infected patients , our result support this synergistic metabolic effect of HCV in development of HCC as the group with IR have more prevalence of cancer . Moreover, **Kawaguchi *et al* [32]** reported that the development of intrahepatic complications, including hepatocellular carcinoma (HCC), is known to be associated with insulin resistance.

Such finding could be explained by hepatic lipid accumulation increases oxidative stress, which may be responsible for the development of HCC **[33]**. Besides these possibilities, insulin has a mitogenic effect, suggesting that insulin may be directly linked to hepatocarcinogenesis **[34].** Also, **Kawaguchi and Sata [32]** added another possible mechanism of IR induced hepatocarcinogenesis as they reported that insulin exerts growth-promoting activity through activation of a mitogen-activated protein kinase pathway; the significant elevated IL-8 in IR group could be explained due to the associated elevated oxidative stress which supposed to be initiator of IL-8 production thus increased oxidative stress could be the link between IL-8 elevation and HCC with IR .

As IL-8 work as proinflammatory cytokine our study try to find is there a relation between this cytokine and HCC with IR and its gene profile .this work reveal a strong significant elevated serum IL-8 level in HCC with IR against without IR(397+/- 176.3 , 269+/- 18.7 respectively) with p value < 0.001.

Linking the genotyping of IL-8 and insulin resistance state can be explained by that the cytokines profile in diabetic patients show marked variability as microvascular complications occur for example in retinopathy and nephropathy and have an incriminated role in its pathophysiology . TNF-α has critical role in development of microvascular complication **[35]** and is also an inducer of IL-8. IL-8 is stimulated in vitro by high glucose concentrations in endothelial cells and has a chemotactic activity for neutrophils and T lymphocyte **[36].** Additionally**, Huseynova *et al* [37]** found increased serum IL-8, TNF-α and TGF-β1 levels in type 2 diabetic patients with a significant increase in the complicated diabetic patients .These data suggest that these cytokines including IL-8 may participate in the development and progression of diabetic complications and have a link to insulin resistance.

Serum IL-8 level used by some investigators for monitoring the clinical course of some cancer types, consistent with its potential role in cancer pathogenesis **[38].** Our work demonstrate highly significant elevated IL-8 in HCC patients than that of healthy control (415.9+/- 213.3 versus control 13.6+/-11.3 with p value <0.001) .this is consistent with studies of **Ren *et al* [9]** and **Morris & Kaplan [39]** , however the new in our work that IL-8 significantly elevated in HCC with IR than those without IR.

Some studies link obesity related complication to interleukin -8 which is a proinflammatory chemokine, secreted from adipocyte and increased with fat mass in animals **[40]** as well as obese subject **[41]** implicating its role in pathophysiology of associated complication; study done by **Kim *et al* [42]** found a close relation between obesity parameters e.g waist circumference ,HOMA and dyslipidemia which in turn conclude a role of IL-8 to obesity complication like atherosclerosis and diabetes .

Our hypothesis in this study suppose a relation between insulin resistance associated with HCC and IL-8 which could explain the carcinogenic invasiveness in these group when compared to HCC without insulin resistance, our result confirm this hypothesis by the significant elevation of serum IL-8 level in HCC with IR in comparison to other group as well as its significant relation to higher BMI, HOMA-IR value level and dyslipidemia .

This could be explained in the light of IL-8, a pro-inflammatory chemokine with a Cysteine-X-Cysteine (CXC) motif, is a soluble mediator secreted from tumor cells that simultaneously exhibits autocrine and paracrine functions within the tumor microenvironment. IL-8 can function not only on leukocyte chemotaxis, inflammatory responses

and infectious diseases but also, on endothelial cells to promote motility, invasion, and the activation of survival and proliferative pathways in mesenchymal and aggressive tumor cells **[43].**
The biological action of IL-8 is mediated through binding to two high-affinity cell surface receptors, CXCR1 (IL-8RA) and CXCR2 (IL-8RB), which are transmembrane G-proteincoupledreceptors. Accumulating studies suggest that IL-8 plays important and multi-functional roles in malignant tumor progression and metastasis **[44].**
In addition, cancer-derived IL-8 promotes not only cell invasion and migration but also, metastasis by inducing neutrophil infiltration and tumor-associated macrophage production of growth factors at the tumor site **[45]**. Furthermore, IL-8 is associated with angiogenesis is in various human tumors, suggesting this might be an important regulatory factor in the tumor microenvironment **[46].**
This is the first study to associate the IL-8 gene -251 T/A and +781 C/T polymorphisms with risk of HCC in Egypt. In 2011, a wider similar study was done by **Chien *et al* [11]** as they studied the association of the IL-8 gene polymorphisms with risk of HCC in the Taiwanese population. They investigated four single nucleoytide polymorphisms including -251 T/A, +781 C/T, +1633 C/T, and +2767 A/T polymorphisms and nearly similar results were found as they reported that the +781 C/T polymorphism significantly associates with incidence of HCC in their work,their data indicated that carriers of the IL-8 +781 T/T homozygote may have decreased risk of HCC. Moreover, they explained the possible relation of IL-8 -251T/A polymorphism by that the common -251 A/T polymorphism of the IL-8 promoter reportedly influences production and expression of IL-8 .Also, they stated that in a meta-analysis study, an association between individuals carrying the IL-8 -251 A/A genotype and a higher tumor risk in the African population, but not in Asian and European populations **[11]**.

2. Conclusion

From the current study, it could be concluded that IL-8(-251 and +781) SNPs can considered to be a candidate gene for the liability of HCC and also, could predict the prognosis of the progression of chronic HCV infection to HCC or not. Moreover, Insulin resistance state and steatosis could be a complicating event of chronic HCV and could be considered as a possible predisposing factor for HCC in such patients inspite of there is found correlation with the studied SNPs in IL-8 gene which need further extended studied to validate these results .
Limitation of the study: the numbers of case and control subjects were relatively small.
Recommendation: Further studies with larger sample sizes are needed to validate the genetic effects of the IL-8 polymorphisms on HCC, along with further collection of serum samples to study the IL-8 sequence variants (-251 and +781) and their biological function in Egyptian patients. Also, to correlate them with the insulin resistance and hepatic steatosis state. Moreover, other single nucleotide polymorphism sites are needed to be subjected for investigation in Egypt.

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