*Research Article*

# The association of interleukin 6 single nucleotide polymorphism with the susceptibility of Egyptians to HCV infection

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## Abstract

**Background:** Hepatitis C virus (HCV) is considered as a main leading cause for cirrhosis and hepatocellular carcinoma (HCC). We aimed to examine the association of IL-6 single nucleotide polymorphisms with the susceptibility of Egyptians to HCV infection. **Patients and methods:** For comparative purposes, four groups were enrolled; chronic HCV (CHC-group, n=22), HCV-related liver cirrhosis (HCV-LC-group, n= 22), HCV-related HCC (HCV-HCC-group, n=54) and an apparently healthy control group (Control-group, n=48). IL-6 rs-1474347 genotyping was performed using real time PCR assays. **Results:** For IL-6 rs1474347, AA genotype was more frequent in CHC, HCV-LC and HCV-HCC compared to controls. **Conclusion:** Screening for IL-6 rs 1474347 AC genotype is considered a good marker for testing the susceptibility of Egyptians to be susceptible for HCV infections and the progression of the disease.

**Keywords:** Hepatitis C virus, cirrhosis and hepatocellular carcinoma

## Introduction

Generally, viral hepatitis is conceded the seventh leading cause of death worldwide. About 50% of death cases were associated with the infection with Hepatitis C virus. According to world health organization (WHO) reports, HCV incidence rate is 3% worldwide. HCV is considered as a primary cause for liver fibrosis, cirrhosis and cancer. As about 10-20% of chronic HCV patients cases will progress to liver cirrhosis within 20 to 30 years and 1-5% of cirrhotic patients can develop hepatocellular carcinoma (HCC) (Obienu et al., 2011, Raphael et al., 2012)

Many and different single nucleotide polymorphism in cytokines genes pro and anti-inflammatory were found to have a significant role in the progression and the outcome of chronic infection with HCV (Mantovani et al., 2008). Interleukin 6 (IL-6) is a pleiotropic cytokine that has both pro and anti-inflammatory action. It plays an important role in many types of cancer and autoimmune diseases such as prostatic, cervical and liver cancer and rheumatoid arthiritis (Liu et al., 2017, Sghaier et al., 2017, Ad'hiah et al., 2018). In addition, IL-6 is known to regulate tumor cell growth and antiapoptosis process and it is secreted by many cells, monocytes, fibroblasts, osteoblasts, keratinocytes, endothelial and mesenchymal cells. On the other hand, elevation of Il-6 was correlated to advanced stages of cancer (Sghaier et al., 2017)

The aim of our study is to determine the association of IL-6 single nucleotide polymorphism with the susceptibility of Egyptian population to HCV infection and their progression.

## Materials and methods

### Study population:

Blood sample were collected from 146 Egyptians attending Assuit university hospital, Egypt. They are categorized into 4 groups. Control group which included 48 healthy individuals (with no signs of liver diseases and negative for HCV and HBV infection), with age ranged from 24 to

48years. The second group included 22 chronic HCV patients with age ranged from 23 to 65 years. The third group included 22 HCV patients who developed cirrhosis with age ranged from 49 to 70 years. The fourth group included 54 HCV patients with HCC with age ranged from 45 to 85 years. Patients with any other viral infections and any liver diseases were excluded.

All patients were clinically diagnosed and confirmed to suffer from chronic HCV, cirrhosis and HCC. Forty-eight healthy controls with no history of previous liver disease (HBV and HCV infection are negative) were included in our study. All the studied groups were free from heart diseases, kidney diseases, muscle disorders, pancreatitis and concurrent HBV, HDV and HIV infection. Patients were tested for aspartate aminotransferase (AST), alanine aminotransferase (ALT), Prothrombin activity, bilirubin, albumin, platelet count, alfa-fetoprotein (AFP) level, hemoglobin (Hb), red blood cells count (RBCs), white blood cells count (WBCs), HIV (anti-HIV), hepatitis B (HBsAg), hepatitis C (anti HCV) serology. In case of reactive anti-HCV positivity, HCV-RNA and viral genotyping tests were performed. HCV patients with cirrhosis were diagnosed depending on the abnormal morphologic findings of liver obtained by computerized tomography (CT), ultrasonography, fibro-scan, laboratory results. Patients with HCC were confirmed by ultrasound, CT, magnetic resonance imaging, histopathology, serum AFP levels more than 400. Also, HCV patients were checked for the presence of ascites and splenomegaly. Response of patients for treatment and the death of any case were listed. All patients and controls gave informed consent for participating in this study.

***DNA extraction***

Peripheral venous blood was collected from participants in EDTA-containing tubes. Total genomic DNA was prepared by the mini-spin column using Thermo Scientific Gene JET Whole Blood Genomic DNA Purification Mini Kit according to the manufacturer instructions. Samples were stored at -80˚C until SNPs' genotyping by real-time polymerase chain reaction (PCR) were carried on.

***IL-6 Single Nucleotide Polymorphisms (SNP)***

One substitution at positions rs-1474347 in IL-6, which was previously reported in the literature with clinical relevance using NCBI Gene SNP Gene view, were tested. Genotyping was performed by allelic (VIC- and FAM-labelled) discrimination method, using assay-on-demand TaqMan assays, which were ordered from Applied Bio-systems (Foster City, CA). The reaction was performed using 7500 Fast Real Time PCR (Applied Biosystems; Thermo Fisher Scientific). Genotypes were assigned from output of clusters, with homozygous major (blue), heterozygous (green) and homo-zygous minor (red) genotypes color-coded. Replicate blinded quality control samples were included to assess reproducibility of the genotyping reaction and concordance was >99%.

## Statistical analysis

IL-6 polymorphisms on gene expression and clinical data were evaluated by setting homozygous major allele genotype as reference; subsequent analyses were done using one-way ANOVA. Chi-square test (or Fisher's exact test for low numbers) was used in comparing categorical data. Logistic regression analysis was used in analyzing the independent contribution of key covariates with HCV outcomes; $P < 0.05$ was considered statistically significant.

## Results

**Patients characteristics:**

The main characteristics of the four groups included in our study were listed in table 1. Significant differences in age among the tested groups were reported. Patients with HCC were elder followed by cirrhosis and chronic HCV patients ($P< 0.00001$). Male sex was the most common in HCC tested group in comparison to the other tested groups. Regarding the liver function test results, it was observed that patients of HCC group showed higher levels of AST and total bilirubin in compassion to

cirrhotic and chronic HCV patients while patients with cirrhosis showed higher average level of ALT.

Significant differences among the tested groups in albumin (P=0.00001) were observed. As lower levels of albumin was found in cirrhotic group followed by HCC patients. Mild and moderate ascites were observed in patients with cirrhosis (63.3%) and HCC (37.3%) while splenomegaly was mostly observed in patients with cirrhosis (77.2%) and 44.4% of HCC patients. The average level of AFP was very high in HCC group in comparison to patients with cirrhosis while normal AFP level was observed in patients with chronic HCV infection and control group. In addition, mortality rat was higher in HCC group (16.6%) in comparison to other tested groups.

**Frequencies and distribution of IL-6 polymorphisms among the tested groups**

Table 2 summarizes the frequencies of the tested SNPs of IL-6 rs1474347. It was found that AA genotype was more frequent in chronic HCV patients (63.8%), followed by cirrhotic group (63.8%) and HCC group (61.1%) in comparison to control (G1) (8.3%) (control vs chronic HCV, HCC P<0.05). In addition, the decrease in A/C genotype resulted in the increase of the patient susceptibility to HCV infection and its progression to cirrhosis and HCC.

**Association of IL-6 rs1474347 with HCV outcomes:**

Our results showed AA genotype was favorable for the progression of HCV infection to chronic or cirrhosis and HCC while AC genotype was non-favorable nor associated with the progression of HCV infection. In addition, patients with IL-6 rs1474347 A/A genotype had 69.6 more chances to develop chronic HCV infection, 19.25 more chances to develop cirrhosis and 17.2 more chances to develop HCC which indicates that A/A genotype play a significant role in the progression of HCV infection. (Table, 3).

**Table (1): Baseline characteristics of chronic HCV patients and chronic HCV patients with cirrhosis and HCC:**

| Variables | Control group (G1) Mean± S.D | Chronic HCV(G2) Mean± S.D | Cirrhosis (G3) Mean± S.D | HCC (G4) Mean± S.D | P-Value |
|---|---|---|---|---|---|
| **Age(years)** | 40.5± 8.4 | 43.13± 16.62 | 58.27± 6.4 | 64.16± 12.106 | < 0.00001 |
| **Male/ female** | 28 (58.3%) /20 (41.6%) | 11 (50%) /11(50%) | 16 (72.7%) /6(27.2%) | 49 (90.7%) /5(9.2%) | G2 Vs G3 0.1216 G2 Vs G4 0.000078 |
| **Hb (g/dl)** | 13.7 ± 2.1 (11.5-14.7) | 12.68± 1.55 (10-15) | 10±1.57 (7-13) | 11.37±2.5 (7-17) | 0.00041* |
| **WBC $10^{3/mm3}$** | 7.2± 0.8 (4-9.6) | 5.6± 1.8 (3-10) | 7.8±4.2 (3-21) | 6.8± 3.07 (3-15) | 0.1643 |
| **Platelet count** | 342±62.1 (220-420) | 202.59 ± 47.9 (148-335) | 138.54 ± 74 (48-383) | 158.14 ± 82.3 (47-403) | 0.013286* |
| **PC** | 90.6±5.2 (85-100%) | 85.4% ± 3.01 (81-95%) | 56.8% ± 18.2 (41-99%) | 72.53% ± 17.5 (45-98%) | < 0.00001** |
| **INR** | 1.15±0.03 (1-1.1) | 1.05 ± 0.051 (1-1.1) | 1.45 ± 0.33 (0.9-1.7) | 1.2 ± 0.177 (1.1-1.6) | < 0.00001** |
| **T BIL(mg/dl)** | 0.1±0.09 (0.1-0.25) | 0.21±0.2 (0.1- 0.6) | 3.4± 2.1 (0.6-8.8) | 2.29±1.2 (0.4- 6.3) | 0.00001** |
| **ALB** | 4.7±0.56 (4-5.2) | 3.95±0.75 (0.8- 4.5) | 2.5±0.5 (1.6-3.5) | 3.07±0.7 (1.7- 4.4) | 0.00001** |
| **AST(U/L)** | 22.1±5.6 (17-28) | 34.7 ± 9.2 (21-62) | 52.6 ± 45.04 (21-221) | 83.5 ± 79.06 (20-405) | 0.00645** |
| **ALT(U/L)** | 21.2±3.9 (14-25) | 31.6 ± 10.7 (21-57) | 50.4 ± 77.1 (6-383) | 41.8 ± 41.7 (15-310) | 0.434 |
| **ASCITES** | | | | | |
| **- NO** | 100% | 22 (100%) | 8 (36.3%) | 34 (62.9%) | 0.000882** |
| **- Mild** | | 0 | 1 (4.5%) | 4 (7.4%) | |
| **- Moderate** | | 0 | 13 (59%) | 16 (29.6%) | |
| **AFP ng/ml (0-11)** | 1.1 ±0.3 (0.5-3.7) | 3.5 ±1.2 (2-5.4) | 8.45±7.43 (2-30) | 785.3± 203.3 (2-12423) | 0.045* |
| **Splenomegaly** | | | | | |
| **Yes** | 0 (0%) | 0 (0%) | 17(77.2%) | 24(44.4%) | < 0.05* |
| **No** | 48 (100%) | 22(100%) | 5(22.7%) | 30(55.5%) | |
| **Treated** | | | | | |
| **No** | - | 11(50%) | 10(45.4%) | 34 (62.9%) | >0.05* |
| **Yes** | | 11(50%) | 12(54.5%) | 20 (37.03%) | |
| **Death** | | | | | |
| **Yes** | - | 0(0%) | 1(4.5%) | 9 (16.6%) | 0.08* |
| **No** | | 22(100%) | 21(95.4%) | 45 (83.3%) | |
| **Response** | | | | | |
| **No** | | 4(18.1%) | 10 (45.4%) | 34 (62.9%) | <0.0001** |
| **SVR** | - | 18(81.8%) | 11 (50%) | 15 (27.7%) | |
| **Relapse** | | 0 (0%) | 1(4.5%) | 5 (9.2%) | |

**Note: All tested patients group were negative for HBsAg and HIV antibodies.**
*** Significant value (p<0.05) and ** highly significant (P<0.0001).**

**Table (II): Genotype frequencies Distribution of IL- 6 rs 1474347 C>A among the tested groups**

| | HCV negative (G1) N= 48 | Chronic HCV (G2) N = 22 | Cirrhosis (G3) N = 22 | HCC (G4) N = 54 | G1 Vs G2 P- Value | G1 Vs G3 P - Value | G1 Vs G4 P- Value | G2 Vs G3 P- Value | G2 Vs G4 P- Value | G3 Vs G4 P- Value |
|---|---|---|---|---|---|---|---|---|---|---|
| **IL-6 rs 1474347** | | | | | | | | | | |
| AA | 4 (8.3%) | 19 (86.4%) | 14 (63.8%) | 33 (61.1%) | **AA versus** | **AA versus** | **AA versus** | **AA versus** | **AA versus** | **AA versus** |
| AC | 40 (83.3%) | 2 (9.09%) | 80 (36.3%) | 20 (37%) | **AC + CC** | **AC + CC** | **AC + CC** | **AC + CC** | **AC + CC** | **AC + CC** |
| CC | 4 (8.3%) | 1 (4.5%) | 0 (0%) | 1 (1.8%) | **< 0.05*** | **< 0.05*** | **< 0.05*** | **0.08** | **0.03*** | **0.837** |

***Significant association P< 0.05, ** highly significant (P< 0.0001).**
**percents were correlated to the total number of each group.**

**Table (III): Association of IL- 6 rs 1474347 C>A SNPs with HCV out comes [chronicity, cirrhosis and HCC]:**

| Variables | HCV negative (G1) Vs Chronic HCV (G2) | | HCV negative (G1) Vs Cirrhosis (G3) Mean± S.D | | HCV negative (G1) Vs HCC (G4) Mean± S.D | |
|---|---|---|---|---|---|---|
| | OR [confidence interval] | P-Value | OR [confidence interval] | P-Value | OR confidence interval] | P-Value |
| **IL-6 rs 1474347** | | | | | | |
| AA | **69.6 [14.19 – 341.8]** | **< 0.0001**** | **19.25 [5.02–73.7]** | **<0.0001**** | **17.2[5.4–55.17]** | **<0.0001**** |
| AC | **0.02 [0.003 – 0.103]** | **< 0.0001**** | **0.1143 [0.0361– 0.3622]** | **< 0.0001**** | **0.1176[0.046–0.3]** | **<0.0001**** |
| CC | - | - | - | | - | |

**OR: Odds ratio, Cl: confidence level, P: Chi-square test between G1 (normal persons) and the other tested groups (patients with chronic HCV (G2), Cirrhosis (G3) and HCC (G4). Significant association P< 0.05, ** highly significant (P< 0.0001).**

## Discussion

Recently, genetic susceptibility of patients to develop different types of cancer has gained attention nowadays. As it was found that there is a relation between the genetic profile of many interleukins and the ability of patient to develop cancer (Aroucha et al., 2016, Sghaier et al., 2017).

Our study showed significant differences among patient with HCV chronic infections, cirrhosis and those with HCC in terms of age, sex, platelet count, AST, Total bilirubin, presence of mild or moderate ascites, AFP levels, splenomegaly and the incidence of mortality rate. As it was found that HCC was more pronounced among elder male patients that may be attributed to some bad habits that worsen the fate of HCV infection such as smoking or drug abuse. In addition, the presence of splenomegaly, ascites and high AFP level was found to be common among HCC patients. On the other hand, high level of Total Bilirubin, low level of albumin was commonly associated with HCV patients with cirrhosis followed by HCC patients. The same findings were shown by many studies (Aroucha et al., 2016, Abd El Salam et al., 2017, Sghaier et al., 2017). IL-6 production was found to be increased in association with HCV infection due to the

alteration of immune response by Viral NS5A and core proteins, up regulating toll-like receptors (TLR4 and TLR2) in B cells that lead to an increased inflammatory response (Feldmann et al., 2006). Regarding the dual capacity of Il-6, it was found that IL-6 sustains the cellular growth and anti-apoptotic activities accompanying chronic inflammation (Ishihara and Hirano, 2002, Smith et al., 2008). The present study showed that Patients with HCC showed high level of IL-6 that indicates their role in progression of HCV infection. Many researchers reported the role of high IL-6 serum level in HCV patients with HCC and the need for IL-6 for the spread of HCC cells beyond the liver depending on the finding of very high level of IL-6 in highly metastatic cell in comparison to IL-6 levels in poorly metastatic cell lines (Reichner et al., 1996, Giannitrapani et al., 2011). It has been shown that mutations that alter the expression of certain antiviral cytokines are predictive of HCV treatment response and disease prognosis and therefore may also be instrumental in the biology of spontaneous clearance (Afdhal et al., 2011). Malaguarnera et al. (1996) reported that several functional and non-functional polymorphic variants were described throughout IL-6 gene and some of these variants were associated with severe chronic HCV infection and HCC. Our results showed significant association between IL-6 rs 1474347 A/A SNP HCV outcomes. As, it was found that patients with IL-6 rs 1474347 A/A SNP had 69.6 (95%CL, 14.19-341.8) more chances to develop chronic HCV infection in comparison to control group, 19.25 (95% CL, 5.02 – 73.7) more chances to develop cirrhosis and 17.2 (95%CL, 5.4 – 55.17) more chances to develop HCC. In addition, we found that low frequency of IL-6 rs 1474347 A/C increase the risk of developping chronic HCV infection and the progression of cases to cirrhosis and HCC in comparison to control group. Many researchers studied the association between IL-6 single nucleotide polymorphisms (SNPS) and HCV infection outcomes due to the importance of IL-6 cytokine role in the infection process and its association with poor quality of life in chronic HCV patient (Abd El Salam et al., 2017, Sghaier et al., 2017, Vieira et al., 2019).

## Conclusion

Screening for IL-6rs 1474347 AC genotype is considered a good marker for testing the susceptibility of Egyptians to be susceptible for HCV infections and the progression of the disease

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