# Interferon-Gamma Inducible Protein-10(IP10) as a Predictor of Early Virologic Response in Chronic Hepatitis C Infected Egyptian Patients Stratified for the Interleukin-28B rs12979860 Genotype

**Mohamed AM Bahgat[1] , Walid A Abdeldayem[1], Heba F Pasha[2], Hala IM Hussein[1], Ibrahim M Ibrahim[1], Ahmed A Abdelmoaty[1]**

*Departments of Tropical Medicine[1] and Biochemistry[2], Faculty of Medicine, Zagazig University*

***Corresponding Author***
*Ahmed Abdelmoaty*

***Mobile:***
*+01001941058*

***Email:****drahmedatya33 @gmail.com*

***Key words:*** *IP10 ;HCV; IL28B rs12979860*

**Background and study aim:** Single nucleotide polymorphisms near Interleukin 28B are strongly associated with favourable treatment response of chronic hepatitis C such as, the homozygous CC at markers12979860. Interferon-Gamma Inducible Protein-10 (IP-10) can be produced by a variety of cells, including hepatocytes. Pre-treatment plasma levels of IP10 are elevated in patients chronically infected with hepatitis C virus of genotypes 1 or 4 who do not achieve early virological response(EVR)to treatment. The aim of this study was to evaluate the rule of adding IP-10 to IL28B rs12979860 Genotype in predicting EVR .

**Patients and methods:** The study enrolled 78 naïve chronic HCV patients who have criteria that met the pegylated interferon plus ribavirin (pegIFN-RBV) treatment for chronic hepatitis C virus (HCV) .IP-10 assay and single nucleotide polymorphisms of the IL28B genotype were performed.

**Results:** Patients with EVR was younger than those without EVR with statistically significant different. Patients with EVR had less elevated liver enzyme ,low viral load and fasting blood sugar than those without EVR with statistically significant difference. 100% patients with out early virological response had A2 activity while 31.7% only of patients with EVR had A2 activity with statistically significant difference .Patients with EVR showed lower level of IP10 and 81.7% of them had CC allele genotype with statistically significant difference when compared to patients without EVR. Patients with CC genotype were associated with lower level of IP10, ALT, AST and also low viral load. Patients with low level of IP10 had lower levels of liver enzyme. Cut off level of IP 10 was<605 pico gram/ml; at this cut off value sensitivity was=100%, specificity was= 96.7% and area under the curve (AUC)= 0.99.

**Conclusion:** IP10 level was lower among responder group. IL28 genotype CC was significantly higher in responders when compared with non responders. .Patients with CC genotype were associated with lower level of IP10 and liver enzymes. Patients with CC genotype were associated also with low viral load.

## INTRODUCTION

Hepatitis C virus (HCV) infects more than 175 million people worldwide **[1]** and is the leading cause of end-stage liver disease as well as the primary indication for liver transplantation in Western countries worldwide **[2]**. Egypt has the highest prevalence of HCV worldwide with 9% countrywide and up to 50 % in certain rural areas **[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 (HCC) and liver transplantation in the country **[4]**.

The treatment based on the combination of peginterferon alpha and ribavirin leads to a sustained virological response (SVR) in 40–50% in patients with HCV genotype 1 and 4 and in 80% of those with genotype 2 and 3 **[5]**. Several baseline and on-treatment variables affect the likelihood of achieving SVR. Older age, advanced stage of fibrosis, African-American ethnicity and HCV-related factors including HCV

genotype 1 and high viral load at baseline predict poor response to antiviral therapy [6]. Furthermore, metabolic factors such as high body mass index (BMI), presence and severity of liver steatosis have been reported as negative predictors of response **[7]**. On the other hand, early on-treatment kinetics of HCV RNA e.g. undetectable HCV RNA at week 4, 12 have a high positive predictive value of SVR **[8]**.

Among the baseline predictors of response, the pre-treatment activation of IFN-stimulated genes (ISG) and the host genetic polymorphisms have been the subject of recent and major studies. Regarding ISG, it has been shown that low levels of intrahepatic and systemic CXC chemokine Interferon-gamma inducible protein 10 kDa (IP-10, or CXCL10)**[9]**, a valid surrogate marker of ISG activation, predict a more pronounced first phase decline of HCV RNA during anti-viral therapy and increased SVR rates **[10]**. On the other hand, several independent studies have consistently shown that single nucleotide polymorphisms (SNPs) near Interleukin28B (IL28B) gene, which encodes the type III interferon are strongly associated with response to treatment of chronic hepatitis C. In particular, the homozygous genotypes TT at marker rs8099917, CC at marker rs12979860 and AA at marker rs12980275 are all associated with favourable treatment outcomes **[11]**.

Interferon-gamma inducible protein 10 kDa is a chemotactic CXC chemokine of 77 AA in its mature form**[12]**. Unlike other CXC chemokines, IP-10 lacks chemotactic activity for neutrophils. Rather, it appears to target T lymphocytes, NK cells, and monocytes through its receptor **[13]**. IP-10 can be produced by a variety of cells, including hepatocytes **[14]**, and it has been implicated in the pathophysiological progression of multiple sclerosis**[15]**, diabetes mellitus **[16]** and HIV **[17]**.In the setting of HCV infection, IP-10 mRNA expression in the liver has been reported to be associated with the presence of lobular necroinflammatory activity in liver-biopsy samples**[14]**.

Recently, baseline pre-treatment plasma levels of CXCL10 are elevated in patients chronically infected with hepatitis C virus (HCV) of genotypes 1 or 4 who do not achieve a sustained viral response (SVR) after completion of antiviral therapy **[10]**. CXCL10 in plasma is mirrored by intrahepatic CXCL10 mRNA and both strikingly predict the first days of elimination of HCV RNA "first phase decline" during interferon/ribavirin therapy for all HCV genotypes **[9]**.

## PATIENTS AND METHODS

This prospective cohort study was carried out in Tropical Medicine Department, Faculty of Medicine, Zagazig University Hospital Egypt during the period from June, 2013 to May, 2015.The study enrolled 78 naïve chronic HCV patients who have criteria that met the pegylated interferon plus ribavirin (pegIFN-RBV) treatment for chronic hepatitis C virus (HCV) infection according to the Egyptian national program for prevention of HCV infection. Patients were selected as systematic random sample.

All subjects were chronic HCV patients of genotype 4 submitted to the standard of care therapy (SOC) of pegylated interferon-alfa 2a (180 ug/week) (Pegasys-Roch-Switzerland) or pegylated interferon-alfa 2b (1.5ug/kg/ per week) (PegIntron-Scheringcorporation-USA) in combination with ribavirin according to weight based criteria (less than 70 kg given 1000 mg daily and more than 70 kg given 1200 mg daily ). Patients included met the criteria of the Egyptian national program for prevention of HCV infection. Male or Female patients between 18-60 years old with Positive anti-HCV and HCV RNA (by PCR test) were included with the following criteria: white blood cell (WBC) >4.000/$mm^3$, neutrophil count >2.000/$mm^3$, platelets >100.000 $mm^3$, Hb >12gm for females and 13gm for males, prothrombin Time <2 seconds above upper limit of normal (ULN), fasting blood sugar not more than115 mg/dl or within 20% ULN (140 mg/dl) and If diabetic, HBA1C <8.5%, albumin >3.5 gm/dl, serum creatinine within normal limit, TSH within normal limit, negative markers for HBV, ANA <1/160, alpha fetoprotein <100 IU /ml, If alpha fetoprotein is >100 IU/ml, C.T is recommended and it should be free from any radiological signs of HCC or advanced cirrhosis. Female patients should be practicing adequate contraception and male patients should have their wives practicing adequate contraception. Patients younger than 18 years and older than 60 years, pregnant and breast-feeding females, decompensated liver cirrhosis patients, alcoholics, addicts, patients under immunosuppressant drugs or corticosteroids, patients with history of organ

transplantation, those with active epileptic seizures, ischemic heart disease within the last 6 months, chronic renal failure, autoimmune diseases, retinal abnormalities, severe psychiatric conditions, BMI >35, uncontrolled diabetes, hypersensitivity to PEG-INF or RBV were excluded from the study.

An informed written consent was taken from each patient before inclusion in this study. All patients with positive HCV RNA PCR were subjected to the following**:** Complete history taking, full clinical examination, routine laboratory investigations including CBC, LFT, KFT, PT, INR, FBS and special laboratory investigations including HBs Ag, ANA, AFP, TSH, antibilharzial antibodies, pregnancy test for females in the child-bearing period, IP-10 assay and single nucleotide polymorphisms of the IL28B genotype. Abdominal ultrasonography was performed for all patients to asses liver, spleen, portal vein diameter, ascites, and both kidneys.

IL28B rs12979860 polymorphism genotyping was performed using polymerase chain reaction with confronting two-pair primers (PCR-CTPP) which was developed by Hamajima et al. **[18]** with a purpose to be simpler than the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). For the detection of serum IP-10, the human cytokine multiplex immunoassay kit (MPXHCYTO- 60K- 06) was used according to the manufacturer's instructions from Millipore (Merck Millipore, Bill-erica, MA,USA) **[19]**.

Patients were followed up at weeks 1, 2, 4 and 8 by the following laboratory tests: CBC, LFT and KFT, TSH and quantitative PCR for HCV RNA at week 12 were further performed.

Patients were divided according to response to SOC therapy into two groups: Group I (Early virological Responders) which included patients who achieved 2 log or greater decline in HCV RNA by week 12 and Group II (Non Early virological Responders) which included patients who failed to achieve at least 2 log decline in HCV RNA by week 12 and their treatment was stopped.

All data were analyzed using SPSS 15.0 for windows (SPSS Inc., Chicago, IL, USA) & MedCalc 13 for windows (MedCalc Software bvba). Continuous data are expressed as the mean ± SD, and the categorical data are expressed as a number (percentage). Continuous data were checked for normality by using Shapiro-Wilk test. Independent Student t-test was used to compare two groups of normally distributed data, Mann-Whitney test to compare non-parametric distributed data between two groups. Categorical data were compared using the Chi-square ($\chi^2$) test**.** Receiver Operating Characteristic (ROC) curve was obtained to calculate the cutoff point for IP10to reach the best compromise in prediction of EVR. The sensitivity, which describes the probability of patients with EVR to have a low level of IPI0 and IL28B (1.0 in binary test) was calculated. The specificity, which describes the probability of patients without EVR to have a high level of IPI0 and other IL28B genotype (0.0 in binary test) was calculated. The positive predictive value (PPV), which describes the probability of that patient with a low level of IPI0 and IL28B has EVR was calculated. The negative predictive value (NPV), which describes the probability of that patient with a high level of IPI0 and other IL28B genotype has no EVR was calculated.

P value <0.05 was considered statistically significant (S), p value< 0.005 was considered highly statistically significant (HS) and p value >0.05 was considered non statistically significant (NS). The results were analyzed by the suitable statistical methods.

## RESULTS

**Table (1):** Demographic data of patients with early virologic response (EVR) versus those without EVR

| Parameter | | No EVR N = 18 | EVR N= 60 | Test | P |
|---|---|---|---|---|---|
| Age | | 42.67 ± 2.3 | 38.700 ± 6.50 | 2.5 | 0.014S |
| Sex | Female | 4 (22.2%) | 26 (43.3%) | 1.06 | 0.3 |
| | Male | 14 (77.8%) | 34 (56.7%) | | |

Patients with EVR were younger than those without EVR with a statistically significant difference.

**Table (2):** Laboratory data of patients with early virological response (EVR) versus those without EVR

| Parameter | No EVR N = 18 | EVR N= 60 | Test | P |
|---|---|---|---|---|
| WbCs( 4_10x$10^9$/l) | 6.44 ± 0.62 | 6.38 ± 0.52 | 0.42 | 0.67 |
| Hemoglobin 13-17 g/dl(men) 12-15(women) | 13.2±.4 | 12.8±.7 | 3.5 | .6 |
| Platelets (150-400$10^9$/l) | 246.22 ± 25.4 | 239.70 ± 23.08 | 1.02 | 0.30 |
| ALT(N=5-35) | 81.33 ± 7.34 | 49.40 ± 9.18 | 13.5 | < 0.001 HS |
| AST (N=5-35) | 72.33 ± 6.51 | 41.33 ± 8.32 | 14.4 | < 0.001 HS |
| Albumin(N=5-35) | 4.72 ± 0.35 | 4.76 ± 0.38 | 0.43 | 0.65 |
| Prothrombin concentration% | 89.78 ± 4.89 | 89.57 ± 4.80 | 0.16 | 0.87 |
| Alkaline phoshatase(50-100 Iu/ml) | 195.78 ± 8.70 | 134.27 ± 6.58 | 0.79 | 0.43 |
| Alphafeto protein(up to 11Iu/ml ) | 3.04 ± 0.34 | 2.88 ± 0.45 | 1.36 | 0.17 |
| PCR (Iu/ml) | (328091-8769543) 876543* | (5432-765432) 7865* | 4.99 | <0.001 HS |
| Fasting blood sugar(70-110mg/dl) | 92.11 ± 18.4 | 82.81 ± 7.00 | 3.24 | 0.002S |
| Serum Creatinine (0.6-1.2mg/dl ) | 0.71 ± 0.09 | 0.69 ± 0.07 | 0.99 | 0.32 |

Patients with EVR had less elevated liver enzyme, low viral load and fasting blood sugar than those without EVR with a statistically significant difference. where * is the median.

**Table (3):** Histopathologic data of patients with early virologic response (EVR) versus those without EVR

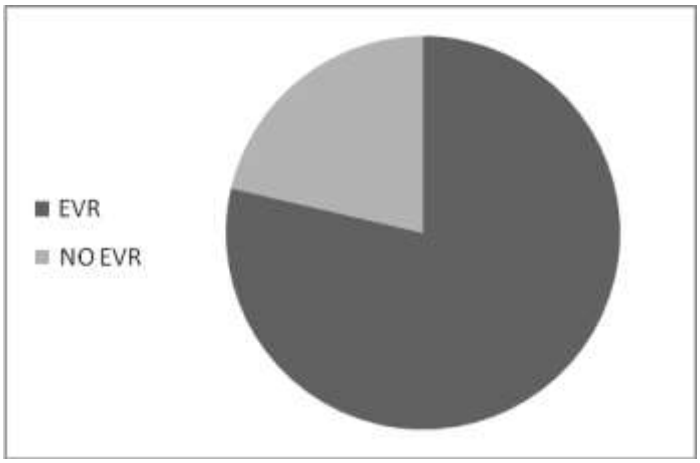
| Parameter | No EVR N = 18 | | EVR N= 60 | Test | P |
|---|---|---|---|---|---|
| Activity | $A_1$ | 0 (0%) | 41 (68%) | 5.8 | 0.15 |
| | $A_2$ | 18 (100%) | 19 (31.7%) | 8.06 | <0.005 s |
| Fibrosis | $F_1$ | 2 (11.1%) | 1 (1.7%) | 0.33 | 0.56 |
| | F2 | 8 (44.4%) | 41 ( 68.3%) | 3.3 | 0.065 |
| | $F_3$ | 8 (44.4%) | 18 (30%) | 3.8 | 0.05 |

100% of patients with out early virological response had A2 activity while 31.7% of patients with EVR had A2 activity with a statistically significant difference.

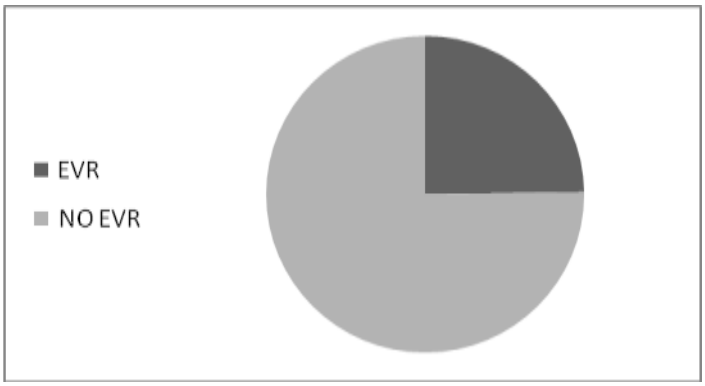
**Table (4):** IP10 and IL28B in the patients with early virological response versus those without EVR

| Parameter | No EVR N = 18 | EVR N= 60z | Test | P |
|---|---|---|---|---|
| IP 10 | 866.67 ± 105.49 | 467.00 ± 90.71 | 15.78 | <0.001 HS |
| IL 28 | | | | |
| CC | 4 (22.2%) | 49 (81.7%) | 22.17 | < 0.001 |
| TT | 4 (22.2%) | 0(0%) | 24.06 | < 0.001 |
| CT | 10 (55.6%) | 11 (18.3%) | 7.95 | 0.004 |

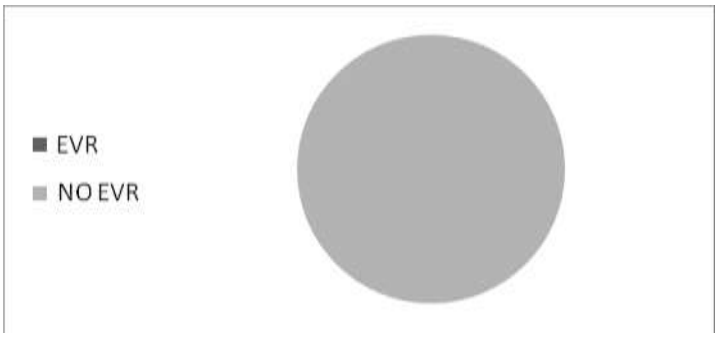
Patients with EVR showed lower level of IP10 and 81.7% of them had CC allele genotype with a statistically significant difference compared to patients without EVR.




A) IL28CC genotype



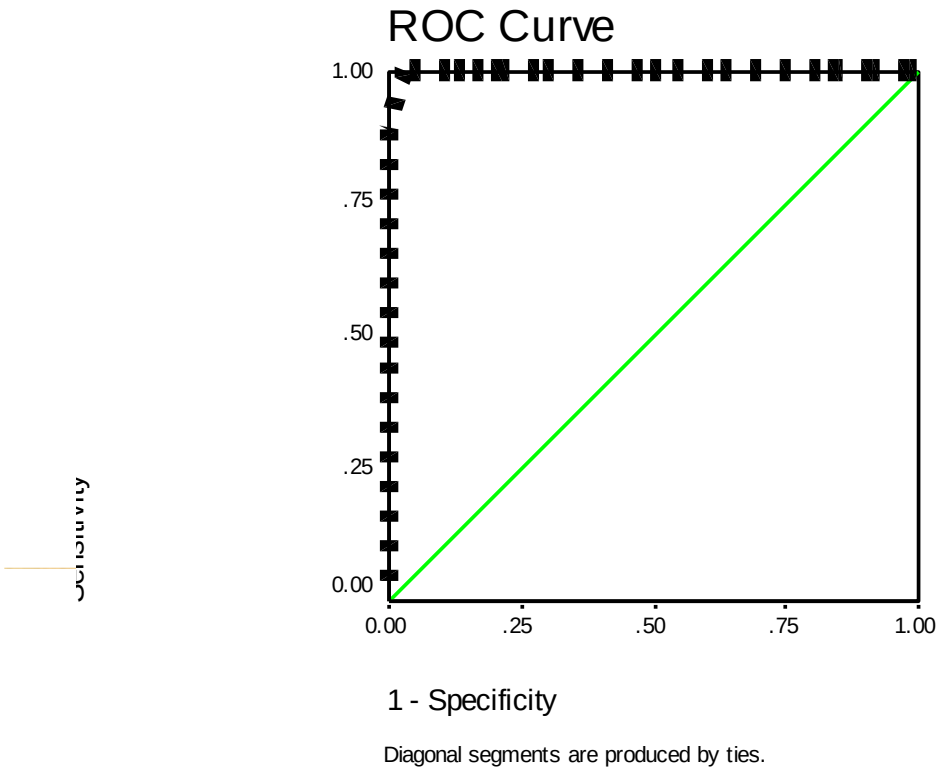

B) IL28CT genotype




C ) IL28TT genotype

**Table (5):** Predictive value for treatment response according to IP10 and IL28B

| Marker | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| IL 28<br>CC | 81.7 % | 77.8% | 92.5% | 56.0% |
| Ip 10<br>Value <605pico gram/ml | 100% | 96.7% | 98.3 | 100% |
| Combination of both IP10 and IL2B CC | 96.7% | 72.22% | 92.1% | 86.7% |




D) I.P 10 area under the curve 0.99 (0.99 – 1.003)

**Table (6):** Different studied parameters in relation to IP10 and IL28B

| IL 28B | CC (53) | CT (21) | TT (4) | F | P |
|---|---|---|---|---|---|
| Ip 10 | 472.45± 139.9 | 704.76±146.3 | 945.0 ± 17.3 | 37.29 | <0.001 |
| ALT | 48.92± 10.9 | 71.66 ± 12.5 | 82.5± 2.8 | 42.01 | <0.001 |
| AST | 40.77± 9.6 | 62.14± 11.2 | 79.0 ± 1.15 | 55.26 | <0.001 |
| PCR | (5432-8769543) 8765* | (65432-1234543) 87654* | (687651-845876) 766763.5 | 0.11 | 0.84 |

Patients with CC genotype were associated with lower level of IP10, ALT, AST and also lower level of HCV RNA level.

**Table (7):** Correlation of IP 10 and some studied data

| Parameter | r | P |
|---|---|---|
| ALT | 0.81 | < 0.001 |
| AST | 0.85 | < 0.001 |
| Alphafeto protein | 0.03 | 0.799 |
| Prothrombin concentration% | 0.12 | 0.27 |
| Albumin | 0.017 | 0.88 |
| PCR | 0.46 | .001 |

Patients with low levels of IP10 had low levels of ALT and AST. also lower level of HCV RNA level.

## DISCUSSION

This study was designed as Egypt has the highest prevalence of HCV worldwide. HCV is a major cause of chronic hepatitis, liver cirrhosis, hepatocellular carcinoma and liver transplantation in the country. Several baseline and on-treatment variables affect the virologic response to treatment of chronic HCV patients as age, stage of fibrosis, HCV genotype and viral load at baseline. Assessment of new markers as IP10 and IL28B will help in prediction of early virologic response. This may be useful in encouraging patients who are difficult-to-treat to initiate therapy.

This study included 78 patients; 76.9% (60 patients) had early virologic response (EVR) while the rest of the patients (23.1 %) had no early virologic response.

We found in our study that patients with EVR were younger than those without EVR with a statistically significant difference and this was in agreement with **[20] [21]**. Also, the efficacy and safety of antiviral therapy in the older population is not clear and are limited to small, single-center studies. Immunological suppression, chronic disease and other medication use in the elderly age group can significantly impair the drug response. However, in patients above 50 years with positive prognostic factors including patients with low HCV RNA levels and those without advanced fibrosis, the SVR rates are comparable with younger patients below 50 years highlighting the importance of other factors in addition to age **[22]**.

In our study we found that patients with low level of ALT and AST has good response to treatment and this result was in agreement with Derbala et al. **[23]**. It was reported that patients with normal liver enzyme have significantly lower inflammation and fibrosis scores on liver biopsy than patients with elevated ALT and the spectrum of liver fibrosis tends to be more severe in patients with elevated ALT than in those with normal ALT. This necroinflammatory activity may affect the response of treatment **[24]**.

Patient with EVR showed lower level of IP10 and 81.7% of them had CC allele genotype with statistically significant different when compared to those without EVR. CC genotype was associated with lower level of proinflammatory mediators in the form of IP10, ALT andASTand patients with low IP10 had low level of enzymes.

We also found high level of IP10 in patients with high level of enzymes and this was in agreement with Zeremski et al. **[25]** who found that the serum IP-10 levels at the time of liver biopsy were predictive of the development of fibrosis 3–5 years later.

Upon HCV infection, IP-10 and other ISGs are produced by hepatocytes and many other cell types. Some ISGs, like IP-10, are produced directly by viral infection without the need for interferon production**.** The relation between ISG expression in response to infection is unknown but clearly relates to the IL28B genotype **[26]**. In chronic HCV, patients with the favourable IL28B genotype tend to have low levels of ISG expression allowing for strong gene induction with therapeutic interferon, lastly leading to clearance. In contrast, those with the unfavourable IL28B genotypes tend to have pre-activation of ISGs with near maximal expression before treatment, resulting in no further gene induction with interferon therapy and thus non-response **[27]**.

If ISG induction is required for clearance, one might have anticipated that in acute HCV infection, patients with higher ISG expression would be more likely to spontaneously clear infection. If plasma IP-10 levels are a reflection of ISG expression, the opposite pattern was seen. However, the relationship between IP-10 and HCV RNA levels may help clarify this apparent paradox. In patients with the favourable IL28B genotype, IP-10 levels tended to be lower but correlated well with the level of HCV RNA suggesting that in this setting, IP-10 production and possibly production of other ISGs is driven by and thus proportionate to the amount of virus present. However, in patients with the unfavourable genotypes, IP-10 levels were on average higher and appeared to be entirely independent of the HCV RNA level. This might suggest that the fundamental problem in those with the unfavourable genotypes is a loss of regulation of ISG induction such that ISGs are produced independent of the stimulus and therefore lead to a less coordinated viral response **[28]**.

Also other studies had shown that IL28B could inhibit HCV replication in a dose- and time-dependent manner and through the JAK-STAT pathway **[29].**So, it had been found that IL28B genotype is associated with differential expression of intrahepatic interferon-stimulated

genes in patients with chronic hepatitis C . All these evidences indicate both anti-viral effect and immune-mediated effect of IL28B, which could be affected by these polymorphisms **[30]**.

**Funding:** None.
**Conflicts of interest:** None.
**Ethical approval:** A written informed consent was taken from all included patients, and the study was approved by the Ethical Committee of our insitution.

## REFERENCES

1. Lauer, G., Walker, B. Hepatitis C virus infection. *N Eng J Med* 2001; 345:41–52.
2. Shepard, C., Finelli,L., Alter M. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005; 5: 558–67.
3. Kamal, S., Nasser, I. Hepatitis C Genotype 4: What We Know and What We Don't Yet Know. *Hepatology* 2008; 47:1371-83.
4. Abdel-Hamid M, El-Daly M, Molnegren V, El-Kafrawy S, Abdel-Latif S, Esmat G., et al. Genetic diversity in hepatitis C virus in Egypt and possible association with hepatocellular carcinoma. *J. Gen. Virol.* 2007; 88: 1526-1531.
5. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis and management, and treatment of hepatitis C: An update. *Hepatology* 2009; 49(4):1335-74.
6. Zeuzem, S. Heterogeneous virologic response rates to interferon-based therapy in patients with chronic hepatitis C: who respond less well? *Ann Intern Med* 2004; 140: 370–81.
7. Bressler BL, Guindi M, Tomlinson G, Heathcote J. High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. *Hepatology* 2003; 38: 639–44.
8. Poordad, F. Review article: the role of rapid virological response in determining treatment duration for chronic hepatitis C. *Aliment Pharmacol Ther* 2010; 31: 1251–67.
9. Askarieh G, Alsiö A, Pugnale P, Negro F, Ferrari C, Neumann AU, et al. Systemic and intrahepatic interferon-gamma-inducible protein 10 kDa predicts the first-phase decline in hepatitis C virus RNA and overall viral response to therapy in chronic hepatitis C. *Hepatology* 2010; 51 (5): 1523–30.
10. Romero AI, Lagging M, Westin J, Dhillon AP, Dustin LB, Pawlotsky JM, et al. Interferon (IFN)-gamma inducible protein-10: association with histological results, viral kinetics, and outcome during treatment with pegylated IFN-alpha 2a and ribavirin for chronic hepatitis C virus infection. *J Infect Dis* 2006; 194: 895–903.
11. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; 461: 399–40.
12. Neville, L., Mathiak, G., Bagasra, O. The immunobiology of interferon gamma inducible protein 10 kD (IP-10): a novel, pleiotropic member of the C-X-C chemokine superfamily. *Cytokine Growth Factor Rev* 1997; 8:207–19.
13. Taub DD, Lloyd AR, Conlon K, Wang JM, Ortaldo JR, Harada A, et al. Recombinant human interferon inducible protein 10 is a chemoattractant for human monocytes and T lymphocytes and promotes T cell adhesion to endothelial cells. *J Exp Med* 1993; 177:1809–14.
14. Harvey CE, Post JJ, Palladinetti P, Freeman AJ, Ffrench RA, Kumar RK., et al. Expression of the chemokine IP-10 (CXCL10) by hepatocytes in chronic hepatitis C virus infection correlates withhistological severity and lobular inflammation. *Journal of Leukocyte Biology* 2003; 74(3): 360-9.
15. Liu, M., Keirstead, H., Lane, T. Neutralization of the chemokine CXCL10 reduces inflammatory cell invasion and demyelination and improves neurological function in a viral model of multiple sclerosis. *J. Immunol*. 2001; 167: 4091.
16. Christen, U., Von Herrath, M. IP-10 and type 1 diabetes: a question of time and location. *Autoimmunity* 2004; 37:273–82.
17. Kutsch O1, Oh J, Nath A, Benveniste EN. Induction of the chemokines interleukin-8 and IP-10 by human immunodeficienc virus type 1 tat in astrocytes. *J Virol* 2000; 74:9214–21.
18. Hamajima N, Saito T, Matsuo K, Kozaki K, Takahashi T, Tajima K. Polymerase chain reaction with confronting two-pair primers for polymorphism genotyping. *Jpn. J. Cancer Res.* 2000; 91: 865–868.
19. Loetscher M, Gerber B, Loetscher P, Jones SA, Piali L, Clark-Lewis I, et al. Chemokine receptor specific for IP10 and MIG: Structure, function, and expression in activated T lymphocytes. *J. Exp. Med* 1996; 184: 963-969
20. Koyama R, Arase Y, Ikeda K, Suzuki F, Suzuki Y, Saitoh S, et al. Efficacy of interferon therapy in elderly patients with chronic hepatitis C. *Intervirology* 2006; 49: 121-126.
21. Terranova R, Luca S. Preliminary results of beta-interferon treatment in the elderly. *Control Clin Trials* 1996; 17: 123-129.
22. Reddy KR, Messinger D, Popescu M, Hadziyannis SJ. Treatment with Peginterferon-alpha 2a and ribavarin in older HCV genotype 1 patients with positive prognostic factors leads to high rates of sustained virological response. *J Hepatol* 2008; 48(Suppl. 2): 310A.

23. Derbala M, Nasser M, Rizk MN, Al-Kaabi S. The predictive value of IL28B rs12979860, rs11881222 and rs8099917 polymorphisms and IP-10 in the therapeutic response of Egyptian genotype 4 patients .*Virology* 2013; 444; 292–300.
24. Shiffman ML, Diago M, Tran A, Pockros P, Reindollar R, Prati D, et al. Chronic hepatitis C in patients with persistently normal alanine transaminase levels. *Clin Gastroenterol Hepatol* 2006; 4: 645–652.
25. Zeremski M, Dimova R, Astemborski J, Thomas DL, Talal AH. CXCL9 and CXCL10 chemokines as predictors of liver fibrosis in a cohort of primarily African-American injection drug users with chronic hepatitis C. *J Infect Dis* 2011; 204: 832-836.
26. McGilvray I, Feld JJ, Chen L, Pattullo V, Guindi M, Fischer S, et al. Hepatic cell-type specific gene expression better predicts HCV treatment outcome than IL28B genotype. *Gastroenterology* 2012; 142: 1122-1131.
27. Feld JJ, Nanda S, Huang Y, Chen W, Cam M, Pusek SN, et al. Hepatic gene expression during treatment with peginterferon and ribavirin: Identifying molecular pathways for treatment response. *Hepatology* 2007; 46:1548-1563.
28. Sarasin-Filipowicz M, Oakeley EJ, Duong FH, Christen V, Terracciano L, Filipowicz W, et al. Interferon signaling and treatment outcome in chronic hepatitis C. *Proc Natl Acad Sci USA* 2008; 105:7034-7039.
29. Zhang L, Miao L, Han F, Dou XG. Cytokine levels in serum of patients with chronic hepatitis C and its significance. *J. Cell. Mol. Immunol.* 2011; 27, 301–303.
30. Urban TJ, Thompson AJ, Bradrick SS, Fellay J, Schuppan D, Cronin KD, et al. IL28B genotype is associated with differential expression of intrahepatic interferon-stimulated genes in patients with chronic hepatitis C. *Hepatology*. 2010; 52 : 1888-1896.

**Peer reviewer: Nahla Elgammal**, Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt. **Editor: Mohamad Emara**, Assistant Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt.