

New Biomarkers for Response to Treatment of HCV Infected Patients Based on IP-10 and IL 28B Polymorphism Analysis

Ahmed Mostafa Aref^{1,*}, Mohamed Shamrouh Othman^{2,3}, Samah Mamdouh⁴,
Ehab Dabaa⁴, Moataz Hassanein⁵, Mohamed Ali Saber⁴

¹Biological Science Department, October University for Modern Sciences and Arts, Egypt

²Preparatory Year College, Hail University, Hail, KSA

³Biochemistry and Molecular Biology Department, Faculty of Biotechnology, October University for Modern Sciences and Arts, Egypt

⁴Biochemistry and Molecular Biology Department, Giza, Egypt

⁵Hepatogastroenterology Department Theodore Bilharz Research Institute, Giza, Egypt

Abstract Single nucleotide polymorphism (SNP)rs12979860 near IL28B gene was shown to be highly predictive of sustained virological response (SVR) in patients with chronic hepatitis C (CHC). Inducible protein 10 (IP-10) in CHC patients increases in non-responders. To assess the potential predictive value of pretreatment IP-10 levels and IL28 B genotype on the SVR in CHC Egyptian patients, eighty-seven patients were included in this study in addition to twenty control subjects. HCV viral load levels were assessed at 0, 12, 24, 48 and 72 weeks, IL28B single nucleotide polymorphism rs12979860 was genotyped and serum IP-10 level were evaluated for each patient. Results have shown that the distribution of SNP was CC (28.1%), CT (43.6%) and TT (28.1%) with SVR 70%, 38.7%, 50% respectively, at cutoff value 342pg/ml for predicting SVR we achieved a sensitivity and specificity of 80% and 46%, respectively. In conclusion, pretreatment serum IP-10 level when added to IL28 genotype, the predictive value is greatly enhanced especially for CT and TT IL28 genotypes.

Keywords Chronic hepatitis C, IP-10, IL28 polymorphism, Interferon, Ribavirin

1. Introduction

Hepatitis C is a global health problem. There were 120–180 million hepatitis C virus (HCV) carriers world-wide, with worldwide prevalence estimated at 3% [1]. Egypt has the highest prevalence in the world, with an estimated >500,000 new infections annually making Egypt the highest endemic country [2] with predominance of subtype 4a [3, 4]. Standard treatment of CHC with Pegylated interferon α -2a (peg-IFN α -2a) and ribavirin results in sustained virological response (SVR) rates of less than 50% in HCV genotype 1 or 4 infected patients, contrasted by SVR rates of 70–90% in HCV genotype 2 and 3 patients [5]. This treatment is not only long and costly, but also there are serious side effects (e.g., GIT symptoms, hematologic abnormalities and adverse neuropsychiatric events) [6].

CXCL10 (also known as IFN- γ -induced protein-10, or IP-10), an IFN- and TNF- α -inducible chemokine that can be

highly expressed by endothelial cells, keratinocytes, fibroblasts, mesangial cells, astrocytes, monocytes, neutrophils, and hepatocytes [7]. Many studies reported that patients with CHC have high systemic levels of IP-10 and the base line levels of IP-10 are higher in patients who don't achieve SVR compared with patients with treatment-induced resolution of HCV infection in HCV genotype 1 patients. These studies suggested that IP-10 not only has a pretreatment predictive value in CHC but can also predict rapid virological response (RVR) [8, 9].

Genome-wide association studies (GWAS), have shown that host genetic factor in the form of single nucleotide polymorphisms (SNPs) near the IL28B gene can predict spontaneous and treatment induced viral clearance in HCV genotype 1 [10, 11]. The mechanisms, how this polymorphisms affect antiviral host responses have remained elusive.

We aimed to evaluate the predictive power of the rs12979860 IL28B SNP and pretreatment IP-10 levels on the sustained viral response in HCV Egyptian chronic patients whom underwent peg-IFN α -2a /ribavirin (800–1200 mg) therapy for 48 weeks.

* Corresponding author:

a_aref23@hotmail.com (Ahmed Mostafa Aref)

Published online at <http://journal.sapub.org/ajb>

Copyright © 2016 Scientific & Academic Publishing. All Rights Reserved

2. Patients and Methods

One hundred and seven individuals were included in this study. All patients and controls gave their informed consent, which was ethically conducted in accordance with the Helsinki Declaration.

Individuals under investigation were grouped into:

Group 1: Normal control group, including 20 apparently healthy volunteer with no evidence of liver disease.

Group 2: CHC group, including 87 patients positive for both HCV-Ab and HCV RT-PCR and negative for HBsAg and anti-HBc.

Ten milliliters of fasted venous blood were taken from patients and controls, the blood was left to clot. Serum was separated by centrifugation and stored at -80°C until further examinations.

2.1. HCV-RNA Quantification

HCV-RNA viremia was quantified by the Abbott Real Time HCVm2000 assay (Abbott Molecular Inc., Des Plaines, Illinois, USA) using ABI Prism 7500 for detection. HCV-RNA levels were assessed at 0, 12, 24, 48 and 72 weeks. The lower detection limit is 12IU/ml.

Patients were classified according to Ghany *et al.* [12] into:

- 1) Early virological responders (EVR) if they achieve seronegativity of HCV RNA at 12 weeks of therapy.
- 2) Sustained virological response (SVR) if serum HCV-RNA was un-detectable 24 weeks after completion of therapy.
- 3) Non responder (NR) patients with reappearance of HCV RNA during therapy.

2.2. IP-10 Quantification

Before starting of therapy, serum IP-10 level was measured in all groups by the commercially available enzyme-linked immunosorbent assay (Quantikine® ELISA R & D Systems, Minneapolis, USA). The minimum detectable level is 1.67 pg/mL.

2.3. Liver Histology

Liver biopsies from all patients were read and scored. The Ishak score 18 according to [13] was used for grade of inflammation and necrosis (range 0–18 and for stage of fibrosis (range 0–6).

2.4. Genotyping of IL28B

DNA samples from patients were genotyped for the IL28B polymorphic marker rs1297860 using the ABI TaqMan allelic discrimination kit and the ABI Prism 7500 (Applied Biosystems Inc, Foster City, CA USA), according to manufacturers recommended protocols Detection System (Applied Biosystems).

TaqMan probes and primers were designed and synthesized by Applied Biosystems Inc. Automated allele calling was performed using SDS software from

Applied Biosystems Inc. Positive and negative controls were used in each genotyping assay. The primers and probes utilized were:

NCBI dbSNP ID rs12979860

Forward primer: TGTACTGAACCAGGGAGCTC,

Reverse primer: GCGCGGAGTGCAATTCAAC,

Vic probe: TGGTTCGCGCCTTC,

FAMprobe: TGGTTCACGCCTTC.

2.5. Biochemical Investigations

Serum levels of AST, ALT, ALP, albumin and Bilharzial antibody were measured for all subjects by the commercially available assays.

2.6. Statistical Analyses

Serum IP-10 levels were correlated with SNP and the clinicopathologic parameters including patients age, sex, viral load, liver histology (stage and grade) and Schistosomal infection.

Statistical analyses were performed using SPSS version 17.0. The following tests were used: Chi-square test (χ^2), Spearman correlation and calculation of the mean value, the probability value (P) was expressed as following: P-value > 0.05: non-significant, P-value ≤ 0.05: significant, P-Value < 0.01: highly significant. Sensitivity, specificity, receiver operating characteristic (ROC) curves, and area under the curve (AUC) were carried out using MedCalc (Mariakerke, Belgium) software.

3. Results

AS shown in Table (1) from eighty-seven patients enrolled in this study, 43 patients have shown SVR (49.4%) with mean age 41.7 years (y) while 44 patients (50.6%) were not responders with a mean age 42.34y. Mean body mass index (BMI) for responders was 27.12kg/m² while it was 30.47kg/m² for NR.

We didn't achieve any significance difference in baseline parameters including AST, ALT, ALP, albumin and Bilharzial Ab between patients who achieved SVR and NR, while there was a statistical difference regarding viral load at baseline (W0) and BMI as shown in table 1.

3.1. IP- 10 Serum Levels

The mean serum levels of IP-10 were significantly higher in CHC patients (493.91 ± 28.60) pg/ml than NC group (90.97±8.62), (p< 0.001). At the same time the mean serum levels of IP-10 were significantly elevated in NR group (586.24± 49.74) compared with SVR group (392.78±29.88), p<0.05, (Table 1, Fig 1).

The ROC curve of IP-10 showed an AUC of 0.679 (95% confidence interval 0.56–0.78). At a cutoff value 342pg/ml for predicting SVR, at this point the sensitivity and specificity were 80% and 46%, respectively with a positive predictive value and a negative predictive value of 69% and 61%, respectively (Fig. 2, Table 3).

There was no significant association between IP-10 and clinicopathologic parameters such as age, sex, viral load at (W0, W12), grade of inflammation, stage, BMI, albumin,

AST, ALT, ALP and Bilharzial infection in SVR and NR patients as shown in table 2.

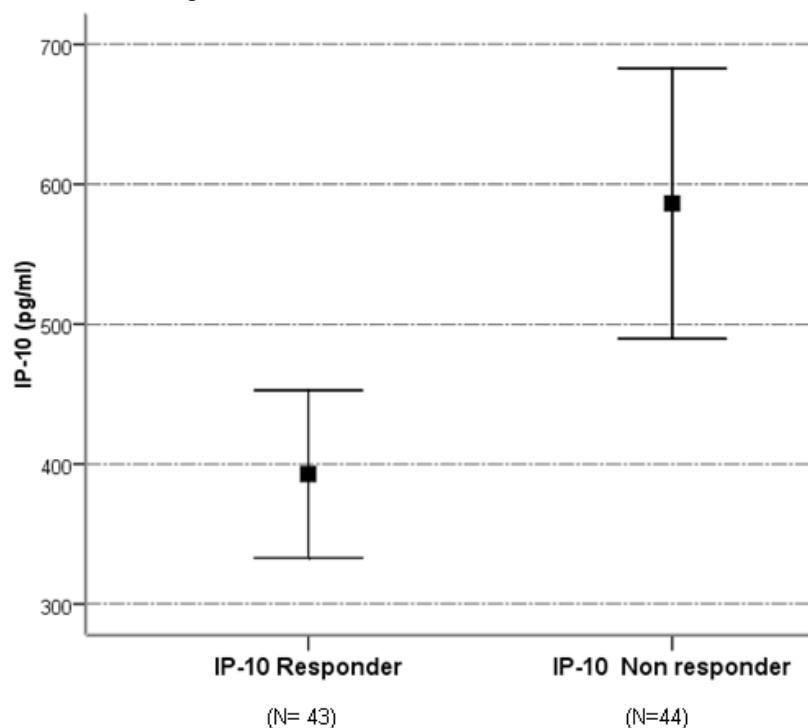


Figure 1. Serum level of IP-10 in SVR and NR patients

Table 1. Patient characteristics at baseline and response to treatment

Variable	Responder		Non Responder		P- Value [†]
	N or (Mean)	% or ± S.E	N or (Mean)	% or ± S.E	
Age (years)	(41.77)	±1.26	(42.34)	±1.29	NS
Sex Male	26	60.5%	23	52.27%	NS
Female	17	39.5%	21	47.73%	
Viral load At W0	(727214.9)	±23365	(1335918)	±29456	0.000**
At W12	0	0	(123554)	±31672	0.037*
Degree Fibrosis 1-2	30	69.76%	34	77.27%	0.807
3-4	13	30.24%	10	22.73%	
Activity 1-3 (Minimal)	30	69.7%	23	52.27%	0.098
4-8 (Mild)	8	18.6%	17	38.64%	
9-12 (Moderate)	5	11.7%	4	9.09%	
13-18 (Severe)	.0	0.	.0	.0	
Bilharzial Ab	(168.2)	±55.36	(219.53)	±60.71	0.51
BMI kg/m ²	(27.12)	±0.59	(30.47)	±0.71	<0.001**
Albumin g/dl	(4.23)	±0.06	(4.13)	±0.06	0.27
ALP (U/l)	(211.97)	±14.84	(226.79)	±77.59	11.69
AST (U/l)	(63.77)	±5.74	(65.84)	±36.68	5.53
ALT (U/l)	(74.15)	±8.17	(70.20)	±43.51	6.55
Serum IP-10 (pg/ml)	(392.78)	±29.88	(586.24)	±49.74	0.005**

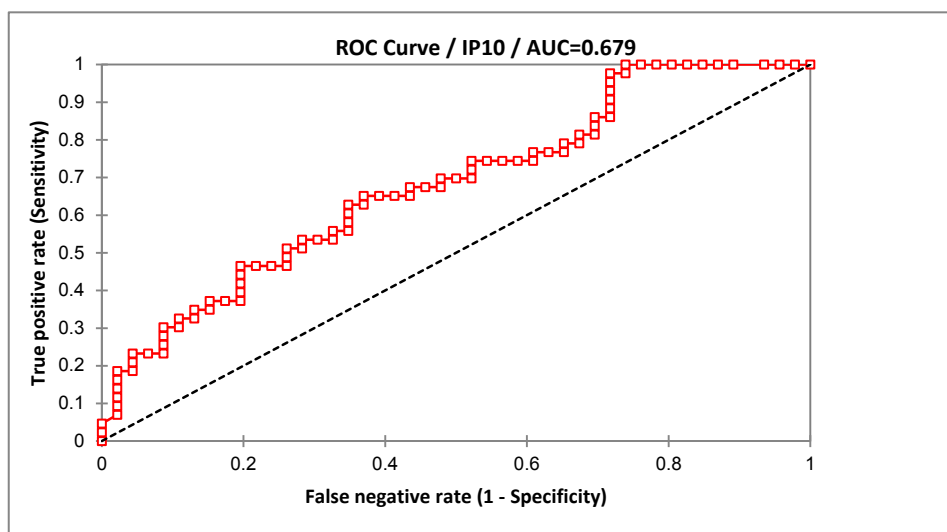
Table 2. Association of IP-10 level with patient characteristics at baseline by response to treatment

Variable [†]	IP10 Responder		IP10 Non Responder	
	Mean	p Value	Mean	p Value
Sex				
male	394.2	0.425	583.6	0.471
Female	390.9		590.1	
Fibrosis				
1-2	376.6	0.425	585.8	0.387
3-4	443.2		625.1	
Activity				
1-3 (Minimal)	379.7	0.415	603.7	0.361
4-8 (Mild)	441.1		611.5	
9-12 (Moderate)	407.3		471.7	
Variable [†]	Corr. Coeff.	p Value	Corr. Coeff.	p Value
PCR				
At baseline	0.05	0.774	-0.09	0.582
After 12 weeks of treatment	.		0.15	
Age	0.04	0.807	0.10	0.513
Bilharzial Ab	-0.26	0.118	0.19	0.224
BMI	-0.13	0.420	0.02	0.910
Albumin	-0.01	0.956	-0.01	0.944
ALP	0.23	0.166	-0.12	0.430
AST	0.12	0.478	0.13	0.826
ALT	-0.06	0.713	-0.14	0.380

Association is tested using chi-square for categorical variables and Spearman correlation for continuous variables.

Table 3. Diagnostic Values of IP-10, viral load and BMI for all patients for discrimination of SVR and non responders to treatment

	Cut -off value	AUC	Sensitivity	Specificity	Accuracy
IP-10	342pg/ml	0.674	80%	46%	80%
Viral load	661,646 IU/ml	0.632	45%	82.5%	63%
BMI	27.4 Kg/m ²	0.716	75%	51%	63.9%

**Figure 2.** ROC curve of IP-10 for discrimination of SVR from NR patients

3.2. HCV Viral Load Results

At base line the mean value for HCV viral load was 727,214 IU/ml in responders while in non-responder patients it was 1,335,918 IU/ml, with statistical significance between the responders and non-responder patients ($p < 0.05$) (Table 1). At week 12 (EVR), HCV-RNA serum level was undetectable in all the responder patients, with statistical significance when compared with NR group ($p < 0.05$).

Figure 3 and table 3, showed that the AUROC for viral load was 0.632, with a sensitivity of 45%, specificity 82.5%, accuracy 63.9% and optimal cutoff value 661,646 IU/ml.

3.3. BMI Results

Table (1) showed a significant decrease in the BMI in SVR compared with NR group ($p < 0.05$).

3.4. Histopathological Results

Early fibrosis stage (score 1-2) was found in 30 (69.7%) of responders versus 34(77.3%) of NR, higher stage (score 3-4) was found in 13(30.2%) of responders and 10(22.7%) of NR patients. In addition the results of this study did not show any statistical significance comparing the grade of necroinflammatory activity with IP-10 even after stratification to minimal, mild and moderate.

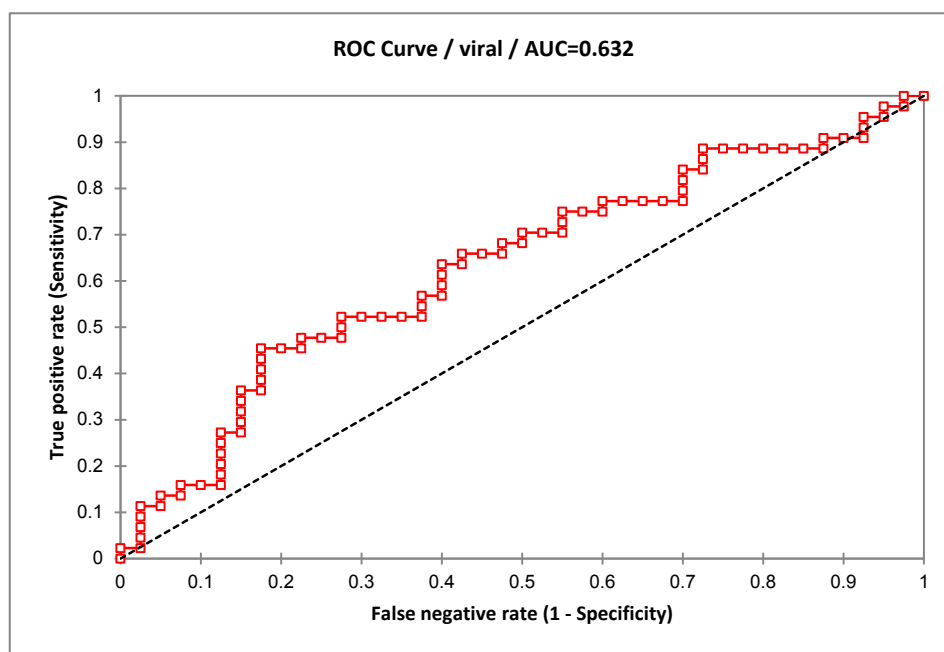


Figure 3. ROC curve of viral load for discrimination of SVR from NR patients

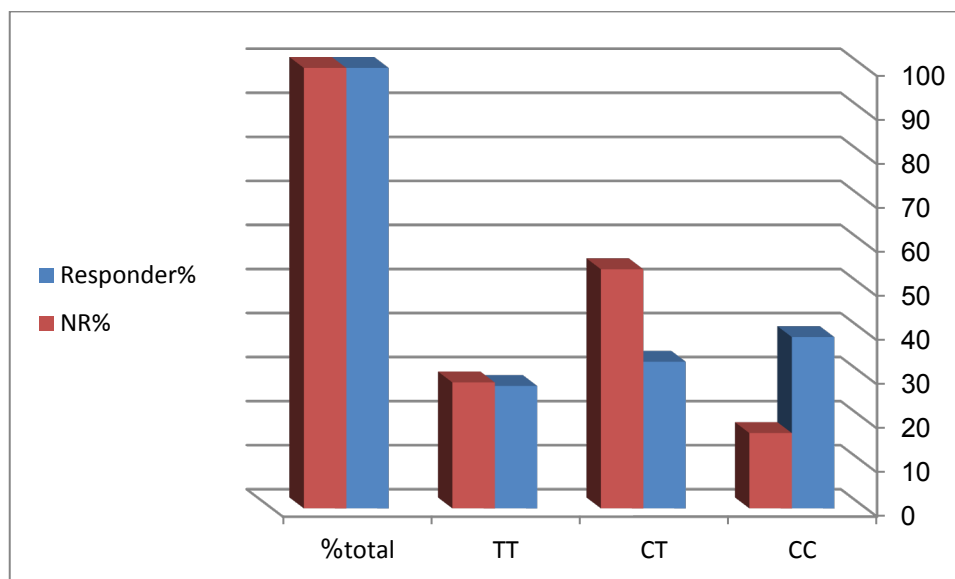


Figure 4. IL28b SNPs genotypes distribution in responder and non responder patients groups

3.5. IL28B Genotype and Treatment Response

The distribution of SNP of IL28 genotypes was CC (28.1%), CT (43.6%) and TT (28.1%) with SVR 70%, 38.7%, 50% respectively as shown in figure (4). There was a significant difference when comparing the CC genotype between SVR and NR patients ($p < 0.05$), but not with CT or TT. Using spearman correlation there was no significance correlation when comparing the genotype with the viral load and IP-10, which indicates that they are independent factors.

4. Discussion

IP-10 is a CXC chemokine that targets T lymphocytes and monocytes, the serum level of IP-10 in CHC patients are higher than healthy controls, rheumatoid arthritis patients and even those infected with HBV patients, these results indicate that IP-10 have a role in the pathogenesis of HCV infection [14-16]. IP-10 is proposed as a predictor factor for SVR for CHC genotype 1 patients pretreatment with interferon and ribavirin [17-19], while only few reports have dealt with genotype 4 [20].

Recently, several reports on a gene polymorphism (rs12979860) upstream of IL28B are favorably associated with treatment response to peg-IFN and ribavirin [21-23]. Carriage of the C allele increases treatment response rates with those who have the CC genotype having the highest SVR rates, CT intermediate and TT have the lowest response [21].

In the current study, we tried to combine the IL28B genotype with baseline IP-10 levels and viral load to demonstrate an independent and additive model for predicting SVR for CHC Egyptian patients treated with the combined peg-IFN and ribavirin.

The main finding of the present study is the significant association of serum IP-10 levels in NR compared to SVR in CHC patients whom underwent peg-IFN- α plus ribavirin therapy showing higher serum IP-10 levels in NR patients compared to SVR patients, 586 and 392pg/ml respectively.

Depending on the ROC curves results of IP-10, at cutoff value 342 pg/ml the best sensitivity and specificity were 80% and 46%, respectively, which could have a discriminating power to differentiate SVR from NR patients. Results also revealed that 37 (84%) NR patients have higher IP-10 than 342pg/ml. these results are inconsistent with many other reports which have shown that baseline IP-10 is lower in SVR patients compared to NR [17, 19, 24, 25].

Our proposed cutoff value is different from that obtained by [18, 19] who have proposed cutoff values at 594 pg/ml and 600pg/ml respectively, to identify genotype 1 HCV responders from non-responders. This higher cutoff value may be due genotype difference, because the predominant subtype in Egypt is 4a [3, 4].

It is unclear why high IP-10 levels are associated with poor response to HCV therapy. The IP-10 receptor (CXCR3) is upregulated on lymphocytes in chronic HCV and hepatocytes appear to be the predominant source of IP-10 in

chronic infection [26-28]. While intrahepatic IP-10 levels correlate with necroinflammatory changes and fibrosis in HCV, its role in viral clearance is less clear. Low pretreatment IP-10 levels are associated with a rapid decline in HCV viral load during the first 24-48 hours of interferon therapy [17].

Patients with low baseline levels of interferon-stimulated genes (ISG) expression appear to have a more robust response to exogenous peg-IFN and a higher SVR rate. In contrast, those with high baseline ISG expression appear to be refractory to further IFN signaling [27, 29]. High IP-10 levels may be a marker of this refractory state, or excess IP-10 may directly interfere with critical signaling pathways.

Casrouge et al. assumed the presence of antagonist form of IP-10, this dominant negative form of the protein, is capable of binding CXCR3 but does not induce signaling, so no cytotoxic T lymphocytes (CTL) migration to the liver, this postulation may support our results regarding the absence of significant association between IP-10 and histopathologic results but this needs further investigations [30].

At base line (W0), there was a significant difference in the viral load between the responders and non-responders ($P < 0.05$). These results are in accordance with other reports that have shown that low viral load is an independent favorable factor for the treatment outcome [24, 31-34]. Our results indicated that 40.9% (18 / 44) of NR patients showed EVR, this means that 29.5% of all EVR patients were relapsed (18 / 61), while 70.5% of CHC patients who showing EVR achieved SVR. Indicating that EVR is a strong predictor of the treatment outcome.

We have observed that 72.2% of the relapsed patients (13 / 18) have serum level of IP-10 above the proposed cutoff value at base line. There wasn't any correlation between IP-10 pretreatment level and viral load at base line and SVR, these results are in accordance with Diago et al. [18] while it was in contrast with Reiberger et al. [24].

Although the serum level of IP-10 is higher in advanced stages of fibrosis, there is no significant correlation between IP-10 and fibrosis. our results are not in accordance with other reports [19, 24, 35, 36] who have shown association between IP-10 and fibrosis in genotype 1 CHC patients and this difference in results may be due dual infection with HIV/HCV [35]. Our finding showed the absence of association of IP-10 and the liver enzymes; AST and ALT. These enzymes are known to be related to the degree of liver damage.

The distribution of IL28 genotype showed that CT is the most frequent genotype (43.6%) followed by CC and TT with almost the same frequency (28.1%). The CC genotype was significantly correlated with SVR in comparison to CT and CC genotypes our results are in consistent with many other reports [22, 23, 37].

In the current study, although the CC genotype have shown the highest SVR (70%), unexpectedly we found that SVR within TT genotype was higher than CT genotype 50% and 38.7%, respectively, which is not in agreement with concept that the T allele is associated with treatment failure.

so we believe that the SNP genotype is not the only factor which is associated with treatment failure.

Combining baseline IP-10 levels with the IL28B rs1297860 genotype provided additional and independent information specifically, with those CT and TT carriers. The overall response rate for CT carriers was only 38.7%, but when we stratified them according to IP-10 cutoff value we found that those with IP-10 levels below 342pg/ml had 61% SVR versus 22% with high IP-10 levels.

For the TT genotype, it showed 50% overall response, with no responders in those whom have IP-10 above the cutoff value while those with low pretreatment IP-10 had 40% SVR. Our results support the results of Darling *et al.* who have shown that the additive value of IP-10 serum levels were most pronounced for those CT and TT carriers [23].

Our results also revealed that there was no any significant correlation between the pretreatment serum levels of IP-10 and IL28 genotypes, indicating that they are independent factors.

5. Conclusions

Predicting response to antiviral therapy is a crucial point in the management of patients with CHC. There is high proportion of Egyptian patients who still do not respond to interferon/ribavirin therapy, so searching for predictive factors in these groups of difficult-to-cure patients is needed. Here we provide evidence that pre-treatment screening of IL28B genetic variants, together with measurement of IP-10 may provide useful information prior to initiating peg-interferon therapy for CHC Egyptian patients.

ACKNOWLEDGEMENTS

This research work was funded by Science and Technology Development Fund (STDF) of Egypt project ID# 1763 (TC/2 Health). We are thankful to Dr. Huda Abo Taleb for her kind assistance in performing statistics.

REFERENCES

- [1] Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005, 5:558-567.
- [2] Miller FD, Abu-Raddad LJ. Evidence of intense ongoing endemic transmission of hepatitis C virus in Egypt. *Proc Natl Acad Sci U S A* 2010, 107:14757-14762.
- [3] Abdel-Aziz F, Habib M, Mohamed MK, Abdel-Hamid M, Gamil F, Madkour S, Mikhail NN, Thomas D, Fix AD, Strickland GT, *et al.* Hepatitis C virus (HCV) infection in a community in the Nile Delta: population description and HCV prevalence. *Hepatology* 2000, 32:111-115.
- [4] Tanaka Y, Agha S, Saady N, Kurbanov F, Orito E, Kato T, Abo-Zeid M, Khalaf M, Miyakawa Y, Mizokami M. Exponential spread of hepatitis C virus genotype 4a in Egypt. *J Mol Evol* 2004, 58:191-195.
- [5] Zeuzem S, Rizzetto M, Ferenci P, Shiffman ML. Management of hepatitis C virus genotype 2 or 3 infection: treatment optimization on the basis of virological response. *Antivir Ther* 2009, 14:143-154.
- [6] Sockalingam S, Blank D, Al Jarad A, Alosaimi F, Hirschfield G, Abbey SE. A comparison of depression screening instruments in hepatitis C and the impact of depression on somatic symptoms. *Psychosomatics* 2011, 52:433-440.
- [7] Luster AD, Ravetch JV. Biochemical characterization of a gamma interferon-inducible cytokine (IP-10). *J Exp Med* 1987, 166:1084-1097.
- [8] Fattovich G, Covolo L, Bibert S, Askarieh G, Lagging M, Clement S, Malerba G, Pasino M, Guido M, Puoti M, *et al.* IL28B polymorphisms, IP-10 and viral load predict virological response to therapy in chronic hepatitis C. *Aliment Pharmacol Ther* 2011, 33:1162-1172.
- [9] Romero AI, Lagging M, Westin J, Dhillon AP, Dustin LB, Pawlotsky JM, Neumann AU, Ferrari C, Missale G, Haagmans BL, *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.
- [10] Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, *et al.* Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009, 41:1105-1109.
- [11] Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, *et al.* Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009, 461:798-801.
- [12] Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009, 49:1335-1374.
- [13] Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, Desmet V, Korb G, MacSween RN, *et al.* Histological grading and staging of chronic hepatitis. *J Hepatol* 1995, 22:696-699.
- [14] Patzwahl R, Meier V, Ramadori G, Mihm S. Enhanced expression of interferon-regulated genes in the liver of patients with chronic hepatitis C virus infection: detection by suppression-subtractive hybridization. *J Virol* 2001, 75:1332-1338.
- [15] Narumi S, Tominaga Y, Tamaru M, Shimai S, Okumura H, Nishioji K, Itoh Y, Okanoue T. Expression of IFN-inducible protein-10 in chronic hepatitis. *J Immunol* 1997, 158:5536-5544.
- [16] Shields PL, Morland CM, Salmon M, Qin S, Hubscher SG, Adams DH. Chemokine and chemokine receptor interactions provide a mechanism for selective T cell recruitment to specific liver compartments within hepatitis C-infected liver. *J Immunol* 1999, 163:6236-6243.
- [17] Askarieh G, Alsio A, Pugnale P, Negro F, Ferrari C, Neumann AU, Pawlotsky JM, Schalm SW, Zeuzem S, Norkrans G, *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, 51:1523-1530.

- [18] Diago M, Castellano G, Garcia-Samaniego J, Perez C, Fernandez I, Romero M, Iacono OL, Garcia-Monzon C. Association of pretreatment serum interferon gamma inducible protein 10 levels with sustained virological response to peginterferon plus ribavirin therapy in genotype 1 infected patients with chronic hepatitis C. *Gut* 2006, 55:374-379.
- [19] Lagging M, Romero AI, Westin J, Norkrans G, Dhillon AP, Pawlotsky JM, Zeuzem S, von Wagner M, Negro F, Schalm SW, et al. IP-10 predicts viral response and therapeutic outcome in difficult-to-treat patients with HCV genotype 1 infection. *Hepatology* 2006, 44:1617-1625.
- [20] Al-Ashgar HI, Khan MQ, Helmy A, Al-Thawadi S, Al-Ahdal MN, Khalaf N, Al-Qahtani A, Sanai FM. Relationship of interferon-gamma-inducible protein-10 kDa with viral response in patients with various heterogeneities of hepatitis C virus genotype-4. *Eur J Gastroenterol Hepatol* 2013, 25:404-410.
- [21] Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009, 461:399-401.
- [22] Khairy M, Fouad R, Mabrouk M, El-Akel W, Awad AB, Salama R, Elnegouly M, Shaker O. The impact of interleukin 28b gene polymorphism on the virological response to combined pegylated interferon and ribavirin therapy in chronic HCV genotype 4 infected egyptian patients using data mining analysis. *Hepat Mon* 2013, 13:e10509.
- [23] Darling JM, Aerssens J, Fanning G, McHutchison JG, Goldstein DB, Thompson AJ, Shianna KV, Afdhal NH, Hudson ML, Howell CD, et al. Quantitation of pretreatment serum interferon-gamma-inducible protein-10 improves the predictive value of an IL28B gene polymorphism for hepatitis C treatment response. *Hepatology* 2011, 53:14-22.
- [24] Reiberger T, Aberle JH, Kundi M, Kohrgruber N, Rieger A, Gangl A, Holzmann H, Peck-Radosavljevic M. IP-10 correlates with hepatitis C viral load, hepatic inflammation and fibrosis and predicts hepatitis C virus relapse or non-response in HIV-HCV coinfection. *Antivir Ther* 2008, 13:969-976.
- [25] Nguyen TT, Niloofar R, Rubbo PA, Nils K, Bollore K, Ducos J, Pageaux GP, Reynes J, Van de Perre P, Tuailon E. Cytokine Response Associated with Hepatitis C Virus Clearance in HIV Coinfected Patients Initiating Peg Interferon-alpha Based Therapy. *Mediterr J Hematol Infect Dis* 2016, 8:e2016003.
- [26] Butera D, Marukian S, Iwamaye AE, Hembrador E, Chambers TJ, Di Bisceglie AM, Charles ED, Talal AH, Jacobson IM, Rice CM, Dustin LB. Plasma chemokine levels correlate with the outcome of antiviral therapy in patients with hepatitis C. *Blood* 2005, 106:1175-1182.
- [27] Sarasin-Filipowicz M, Oakeley EJ, Duong FH, Christen V, Terracciano L, Filipowicz W, Heim MH. Interferon signaling and treatment outcome in chronic hepatitis C. *Proc Natl Acad Sci U S A* 2008, 105:7034-7039.
- [28] Zeremski M, Petrovic LM, Chiriboga L, Brown QB, Yee HT, Kinkhabwala M, Jacobson IM, Dimova R, Markatou M, Talal AH. Intrahepatic levels of CXCR3-associated chemokines correlate with liver inflammation and fibrosis in chronic hepatitis C. *Hepatology* 2008, 48:1440-1450.
- [29] Feld JJ, Nanda S, Huang Y, Chen W, Cam M, Pusek SN, Schweigler LM, Theodore D, Zacks SL, Liang TJ, Fried MW. Hepatic gene expression during treatment with peginterferon and ribavirin: Identifying molecular pathways for treatment response. *Hepatology* 2007, 46:1548-1563.
- [30] Casrouge A, Decalf J, Ahloulay M, Lababidi C, Mansour H, Vallet-Pichard A, Mallet V, Mottez E, Mapes J, Fontanet A, et al. Evidence for an antagonist form of the chemokine CXCL10 in patients chronically infected with HCV. *J Clin Invest* 2011, 121:308-317.
- [31] Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., Haussinger D, Diago M, Carosi G, Dhumeaux D, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002, 347:975-982.
- [32] Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H, Jr., Bernstein D, Rizzetto M, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004, 140:346-355.
- [33] Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001, 358:958-965.
- [34] Zeuzem S, Feinman SV, Rasenack J, Heathcote EJ, Lai MY, Gane E, O'Grady J, Reichen J, Diago M, Lin A, et al. Peginterferon alfa-2a in patients with chronic hepatitis C. *N Engl J Med* 2000, 343:1666-1672.
- [35] Berenguer J, Fernandez-Rodriguez A, Jimenez-Sousa MA, Cosin J, Zarate P, Micheloud D, Lopez JC, Miralles P, Catalan P, Resino S. High plasma CXCL10 levels are associated with HCV-genotype 1, and higher insulin resistance, fibrosis, and HIV viral load in HIV/HCV coinfecting patients. *Cytokine* 2012, 57:25-29.
- [36] Domagalski K, Pawlowska M, Kozielowicz D, Dybowska D, Tretyn A, Halota W. The Impact of IL28B Genotype and Liver Fibrosis on the Hepatic Expression of IP10, IFI27, ISG15, and MX1 and Their Association with Treatment Outcomes in Patients with Chronic Hepatitis C. *PLoS One* 2015, 10:e0130899.
- [37] Youssef SS, Abbas EA, Abd El Aal AM, Omran MH, Barakat A, Seif SM. IL28B rs 12979860 Predicts Response to Treatment in Egyptian Hepatitis C Virus Genotype 4 Patients and Alpha Fetoprotein Increases Its Predictive Strength. *J Interferon Cytokine Res* 2014, 34: 505-509.