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INTERFERON INDUCIBLE PROTEIN-10 LEVEL AND IL28B GENE POLYMORPHISM AS PREDICTORS OF THE RESPONSE TO PEGYLATED INTERFERON / RIBAVIRIN THERAPY IN EGYPTIAN HCV PATIENTS

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ABSTRACT: *Hepatitis C virus (HCV) infection is a major cause of chronic liver disease and hepatocellular carcinoma worldwide. The highest prevalence of HCV infection was reported to occur in Egypt. It is crucial to determine the predictors of Sustained Viral Response (SVR) to Pegylated Interferon (peg-INF) / Ribavirin (RBV) therapy in chronic HCV Egyptians, in order to select the patients who will get benefit from this costly therapy that has frequent side effects. Pretreatment serum interferon Inducible Protein-10 (IP-10) level measurement and genotyping for IL28B rs12979860 polymorphism, were carried out on 82 Egyptian chronic HCV patients receiving peg-INF/ RBV dual therapy for 48 weeks. It was revealed that patients with SVR had lower baseline IP-10 level. The baseline IP-10 level with the best sensitivity and specificity for identifying SVR was 499.02 pg/ml, with 100% specificity and 82% sensitivity. Moreover, the response rates to this dual therapy were 86.2%, 52.9%, 0.0% for genotypes CC, CT, and TT of the IL28B rs12979860 polymorphism respectively. So it can be said that carriage of a C allele is favorably associated with treatment response. Also a statistically significant lower serum IP-10 baseline level was found in the homozygous carriers of favorable CC genotype as compared with carriers of CT and TT genotypes. In conclusion, baseline serum IP-10 and genotyping for IL28B rs12979860 polymorphism are of significant value in predicting the response to peg-INF/ RBV dual therapy in Egyptian chronic HCV infection patients*

KEYWORDS: HCV, IP-10, IL28B, sustained virological response, Interferon, ribavirin.

INTRODUCTION

Hepatitis C virus (HCV) infection is considered the major cause of chronic liver disease and hepatocellular carcinoma worldwide [1]. Egypt has high prevalence of HCV antibodies in the adult population (15–20%), which has been caused by mass parenteral antischistosoma therapy given in the 1960s and 1970s [2]. Genotype 4 is considered the most common genotype of HCV in the Middle East and Africa, especially Egypt which has more than 90% of HCV infections due to genotype-4 [3]. .The goal of treating chronic HCV patients is to eradicate the virus or, in a clinical term, to attain a sustained virological response (SVR). SVR means undetectable HCV RNA at twenty four weeks after treatment cessation [4]. Weekly injections of pegylated interferon-α (peg-IFN) in

combination with daily oral ribavirin (RBV) for 24-48 weeks are used for treating chronic HCV infection [5,6]. However this approved therapy has many unpleasant side effects and is only effective in a certain proportion of chronic HCV patients [7]. Therefore, identifying baseline and on- treatment factors that provide good prediction of SVR in chronic HCV patients is important to avoid unnecessary side effects, to save medical costs and to increase efficacy [8].

Chemokines and cytokines are considered attractive biomarkers for treatment outcome as they are regulators of inflammation and immunity in HCV infection. Many are influenced by exogenous interferon and play important roles in clearance of the virus. The immune system of responders tends to have a lower baseline activation before starting treatment that is markedly induced in response to treatment by IFN [9]. Interferon-gamma inducible protein 10 kDa (IP-10); also called CXCL10 is considered a chemotactic CXC chemokine formed in its mature form of 77 amino acids [10]. IP-10 is produced by variable cells, including hepatocytes [11]. IP-10 acts on the CXCR3 receptor, however unlike other CXC chemokines, doesn't have sufficient chemotactic activity for neutrophils and instead attracts monocytes, T lymphocytes and natural killer cells to the infection sites [10]. Several studies have reported that the non-ELR CXC chemokine IP-10 may be a good prognostic marker for the outcome of HCV treatment in infection with genotype 1 [12]. Elevated IP-10 levels before initiating treatment correlate with non-response to peg-IFN and RBV therapy, although this correlation has not been proven in African American patients [13].

Recently, several genome-wide association studies have detected that single nucleotide polymorphisms (SNPs) in the 19q13 region, that are in close proximity to three genes (IL28A, IL28B, and IL29) which encode cytokines of the IFN-l (i.e. type III IFN) family, predict spontaneous hepatitis C viral clearance [14] as well as achieving SVR after peg-IFN/RBV therapy in HCV genotype 1 patients [15]. Three of these SNPs were reported to be highly predictive of a favorable response to treatment in HCV genotype 1 infected patients: rs12979860 [16], rs12980275 [15], and rs8099917 [17]. Therefore, assessment of both pretreatment IP-10 and IL28B-related SNPs may be useful in response prediction in chronic HCV infection. The current study aimed at evaluation of the value of pretreatment serum IP level and the IL28B rs12979860 SNP in the prediction of the likelihood of SVR in Egyptian chronic HCV patients receiving peg-IFN/RBV dual therapy.

PATIENTS AND METHODS

Eighty two Egyptian adult patients with chronic active hepatitis C were included in this study. They were referred from Viral Hepatitis Center, Benha City-Egypt to the Molecular Biology Unit, Faculty of medicine, Benha University for HCV load assessment by real time PCR. These patients received peg-IFNα-2a (Roche) and RBV for a period of 48 weeks from June 2011 to January 2013 . peg-IFNα-2a was given in a dose of 180µg/week subcutaneously and RBV was given in a daily dose of 1000 mg for patients < 75kg and 1200 mg daily for those at least 75kg for a total of 48 weeks. All patients were followed up for another 24 weeks after the completion of treatment. All patients had compensated liver disease, candidates for treatment with peg-IFNα-2a/RBV for hepatitis C. They fulfilled the inclusion and exclusion criteria of the

national committee for control of viral hepatitis in Egypt [18]. For all patients, liver biopsy was taken and pretreatment measurement of the body mass index (BMI) using the following equation: BMI (kg/m^2) = Weight / Height, was performed [19].

Sampling

Baseline venous blood samples of about 5ml each were collected prior to initiation of treatment. Two ml were put into sterile vaccutainer tube containing EDTA for later genotyping of the IL-28B rs12979860 SNP. The remaining 3 ml were left for clotting and centrifuged for 10 min at 5000 rpm to separate the serum for quantitation of HCV RNA using real time PCR, quantification of IP-10 by ELISA technique, measurement of serum ALT and AST and detection of anti-schistosomal antibodies.Moreover, venous blood samples of about 3ml each were collected in sterile vaccutainer tubes 12 and 24 weeks after the treatment completion, to separate the serum for quantitation of HCV RNA.Both blood and serum were stored at -80°C until further processing.

Absolute quantitation of serum HCV RNA

Absolute quantitation of serum HCV RNA (IU/ml) by RT-PCR using taqman technology was performed at baseline, weeks: 12 and 24 weeks after the completion of treatment.Automated extraction of viral RNA from serum was performed in the QIAcube automated extractor, Qiagen -Germany using QIAamp Viral RNA Mini Kit, Qiagen-Germany, according to the manufacturer instructions. Absolute quantitation of HCV RNA was performed in ABI7900 Real time PCR, Applied Biosystem-USA using Artus HCV RG RT-PCR, Qiagen-Germany, according to the manufacturer instructions.

Patients with undetectable HCV RNA in serum 24 weeks after the completion of therapy were considered Sustained virological responders (SVRs), while those who failed to achieve a reduction of 2 log HCV RNA IU/ml after 12 weeks treatment period or who never achieved undetectable HCV RNA during at least 24 week treatment period were considered Non responders (NRs) [20].

Measurement of serum IP-10 concentration by ELISA technique

This was done using Human (IP10) ELISA Kit; SunRed, Shanghai according to the manufacturer's instructions. Absorbance was measured at 450 nm on an ELISA reader (Infinite F50; TECAN, GmbH).

Genotype determination of IL-28B SNP rs12979860

Genotyping for the IL-28B SNP rs12979860 was done using a PCR based restriction fragment length polymorphism (PCR-RFLP) assay. The amplification of the target genomic region that contains the SNP rs12979860 was done using direct amplification kit (KAPA Blood PCR kits; KAPA BIOSYSYTEM®). It is designed for amplification of DNA fragment directly from whole blood. The primers utilized were: NCBI dbSNP ID rs12979860 forward primer; 5′CCTAACCTCTGCACAGTCT-3′ and NCBI dbSNP ID rs12979860 reverse primer; 5′GCGCGGAGTGCAATTCAA-3′. PCR amplification was carried out using 2.50 µl of each primer, 2.50 µl Tween-20 (2%), 25µl KAPA Blood PCR Mix B, 5µl EDTA blood and PCR grade water Up to 50 µl. Amplification was done in G storm thermal cycler, UK according to the following PCR reaction conditions: initial denaturation at 95 °C for 5 min, followed by 35 cycles of: denaturation at 95 °C for 30 sec., annealing at 52 °C for 30 sec., extension at 72 °C for

30 sec and final extension at 72 °C for 1min. The PCR product for rs12979860 was 110 base pairs.

Ten µl of the PCR reaction mixture was digested with 10 units of the Bsh12361(BstU-I) restriction endonuclease (Thermo scientific) in a total volume of 31 µl at 37°C for 2.5 hours then Bsh12361 was inactivated by incubation at 65°C for 20 min. The DNA fragments and 1000bp DNA ladder were separated on 2% Agarose gel electrophoresis after staining with ethidium bromide. The DNA bands were photographed and analyzed using digital camera 8 megapixel and Photos were analyzed using Gel Documentation System (Alpha Inotech, USA).

Measurement of serum ALT, AST and detection of anti-schistosomal antibodies

Serum ALT and AST were measured colorimetrically using kits supplied by Spinreact, Spain. Detection of anti-schistosomal antibodies was done by Indirect Heme-Agglutination assay (IHA), using Schisto-IHA-Fast^{TM} kit for detection of bilharzial antibodies in human serum supplied by ABC diagnostics, Egypt.

This study was approved by the National Committee for Control of Viral Hepatitis of Egyptian Ministry of Health, Egypt and by the Ethical Review Committee of Faculty of Medicine, Benha University. The research objectives were explained to each patient and a written informed consent was taken from all patients.

Statistical analysis

Analysis of the current study data were done using SPSS version 16. For quantitative data, mean and standard deviation (±SD) were calculated, while Student's t-test and F test (ANOVA test) were used as tests of significance for comparing 2 groups and more than 2 groups respectively. For qualitative data, frequency and percentage were calculated and chi square test (X2-value) or fisher exact test were used as tests of significance . Pearson's correlation coefficient was used to find relationship between different variables. ROC curve (Receiver operator characteristics) determined the cutoff values of serum IP-10 with the optimum sensitivity and specificity for detection of patients with SVR and NRs. P value <0.05 was considered statistically significant (S).

RESULTS

Baseline patients characteristics

Baseline patient characteristics of this cohort are presented in Tables (1,2).

Table (1): Mean values ± SD of quantitative baseline patient characteristics.

Variable	Mean ±SD (Range)
Age (years)	36.41 ± 9.67 (19-53)
BMI (Kg/m²)	25.87 ± 2.23 (22.4-29.8)
Initial baseline HCV-RNA (IU/ml)	434000 ± 437100 (790-2680000)
AST (U/L)	33.44 ± 11.35 (15-71)
ALT (U/L)	40.34 ± 14.98 (12-85)
IP-10 level (pg/ml)	434.41 ± 233.87 (223.7-1107.0)

Table (2): Number and percentage of qualitative baseline patient characteristics.

Variable	N (%) 82 (100%)
Gender:	
Male	31(36.6%)
Female	51(62.2%)
Liver Biopsy [Metavir score degree of histological (necroinflammatory) activity (A) and fibrosis (F)]**:**	
A1F2	51(62.2%)
A2F2	26 (31.7%)
A2F3	5 (6.1%)
Anti-schistosomal Antibodies:	
Positive	48(58.5%)
Negative	34(41.5%)

Response to treatment and its relation to baseline patient characteristics including serum IP-10 level

In this cohort, 59 patients (72%) were SVRs and 23 (28%) were non-responders.

Patients with SVR were significantly younger, had a lower BMI, lower AST and ALT levels, lower IP-10 level but no significant difference in the initial baseline HCV-RNA level was found between SVRs and NRs (Table 3).There was a highly significant association between response to treatment and gender with female patients achieving higher SVR rates than males. Also, there was a highly statistically significant association of treatment response with necro-inflammatory activity and fibrosis stage. Patients with mild activity (A1) and low fibrosis stage (F2) achieved higher SVR rates. On the other hand, there was non significant association between treatment response and the Anti-schistosomal Antibodies presence (Table 4).

Table (3): Mean values ± SD of quantitative baseline patient characteristics in relation to Peg- IFN/ RBV dual therapy response.

Groups / Variable	SVR N=59	NR N=23	Student t test "t"	P value
	Mean ± SD (Range)			
Age (years)	34.95±9.90 (19-53)	40.17±8.08 (23-52)	2.25	<0.05
BMI (Kg/m²)	25.19±1.76 (22.4-29.4)	27.62±2.38 (23.1-29.8)	5.08	<0.01
AST (U/L)	30.22±10.6 (15-71)	41.7±8.9 (23-60)	4.59	<0.01
ALT (U/L)	34.46±10.11 (12-69)	55.43 ± 15.03 (27-85)	7.31	<0.01
Initial baseline HCV-RNA (IU/ml)	450000±333964.3 (790-1301000)	392000±637738.5 (4600-2680000)	0.539	>0.05
IP-10 level (pg/ml)	318.18±79.52 (223.7-496.9)	732.57±235.67 (320.8-1107)	11.96	<0.01

Table (4): Qualitative baseline patient characteristics in relation to Peg-IFN/ RBV dual therapy response

Response / Variable	SVR N=59	NR N=23	X2 test	FET	p value
Gender					
Male	15 (25.4%)	16 (69.6%)	13.71	-	<0.01
Female	44 (75.9%)	7 (30.4%)			
Anti-schistosomal Antibodies					
Positive	32 (54.2%)	16 (69.6%)	1.602	-	>0.05
Negative	27 (45.8%)	7 (30.4%)			
Liver biopsy					
A1F2	44 (74.6%)	7 (30.4%)	-	13.89	<0.01
A2F2	12 (20.3%)	14 (60.9%)			
A2F3	3 (5.1%)	2 (8.7%)			

Different cutoff points of the baseline serum IP-10 level for the likelihood achieving SVR among patients were determined and evaluated (Table 5). The best cutoff point was 499.02 pg/ml as it had 82.6% sensitivity, 100% specificity with accuracy 95.1%.

Table (5): Baseline serum IP-10 level cut off points and their sensitivity, specificity, positive and negative predictive values for the likelihood of achieving SVR in chronic HCV patients.

AUC	0.97	0.97	0.97
Cut off point	371.6	499.02	607.4
Sensitivity	95.6%	82.6%	65.2%
Specificity	79.7%	100%	100%
PPV	64.7%	100%	100%
NPV	97.9%	93.7%	88.1%
Accuracy	84.1%	95.1%	90.2%

It was found that 82.6% of the non responders had a serum IP-10 basal level greater than 499.02 pg/ml and all the responders had a serum IP-10 basal level ≤ 499.02 pg/ml (Table 6).

Table (6): Response of treatment in relation to the best cut off point of 499.02 pg/ml of IP-10.

Response / IP-10 level (pg/ml)	NR	SVR	Total	FET	P value
Positive (>499.02)	19 (82.6%)	0 (0.0%)	19 (23.2%)	58.9	<0.01
Negative(≤499.02)	4 (17.4%)	59 (100%)	63 (76.8%)		

Different correlations of baseline serum IP-10 level with other qualitative and quantitative baseline patients characteristics

This study revealed a positive significant correlation between baseline serum IP-10 level and age, BMI, fibrosis stage and necro-inflamatory activity. However, a non-significant positive correlation was revealed between serum IP-10 level and pretreatment serum ALT, AST and HCV RNA levels (Table 7).

Table (7): Correlation coefficient (r) between serum IP-10 basal level and different variables**.**

IP10 level / Variable	Pearson correlation coefficient (r)	P value
Age	0.302	<0.05
BMI	0.352	<0.01
AST	0.189	>0.05
ALT	0.091	>0.05
Fibrosis stage	0.489	<0.01
Necro-inflammatory activity	0.268	<0.05
HCV RNA	0.154	>0.05

Genotypes of IL-28B SNP rs12979860 and their relation to response to treatment

Of the 82 patients genotyped, 70.7% of genotyped patients were CC, 20.7% were CT and 8.5% were TT. Gel electrophoresis of the target genomic region that contains the rs12979860 SNP after digestion with Bsh12361 restriction endonuclease showed a band of 110 bp that indicated the T/T genotype or 2 bands of 90 bp and 20 bp length that indicated the C/C genotype or 3 bands of 110 bp, 90 bp and 20 bp length that indicated the C/T genotype. However, the 20bp band is usually invisible on the gel, so only one band of 90bp indicated C/C genotype and 2 bands of 110 bp and 90 bp indicated the C/T genotype (Figure 1).

Figure (1): Agarose gel electrophoresis showing different genotypes of IL28B rs12979860 SNP. Lane M showing the DNA ladder. Lanes 1&2 showing 2 bands (110 bp, 90 bp) of C/T genotype. Lane 3&4 showing one band (90bp) of C/C genotype. Lane 5 showing one band (110 bp) of T/T genotype.

There was a statistically significant association between IL28B SNP rs12979860 genotypes and treatment response; high SVR rates among homozygous carriers of CC genotype were observed (Table 8).

Table (8): Relationship between treatment response and genotype of IL28B SNP rs12979860.

Response / Genotyping	SVR N=59	NR N=23	FET	p value
CC	50 (84.7%)	8 (34.8%)	24.82	<0.01
CT	9 (15.3%)	8 (34.8%)		
TT	0 (0.0%)	7 (30.4%)		

Relation of serum IP-10 baseline levels with genotypes of IL28B SNP rs12979860

There was a statistically significant lower serum IP-10 baseline levels observed in the homozygous carriers of the favorable CC genotype (P=0.001) as compared to the carriers of one (CT genotype) or two copies (TT genotype) of the risk alleles (Table 9).

Table (9): Relationship between baseline serum IP-10 levels and IL28B SNP rs12979860.

Genotyping	IP 10 level pg/ml Mean ± SD (Range)	F test	p value
CC	344.2±121.8 (224.4-869.3)	31.15	<0.01
CT	576.02±283.21 (223.7-1107)		
TT	838.01±240.86 (320.8-1096)		

DISCUSSION

In the peg-IFN/ribavirin dual therapy, predictors of response (likelihood of achieving a SVR) can help to decide whether or not to begin the treatment or . Patients having poor predictors of response with mild disease of the liver may be candidates for treatment by the new generations of antiviral drugs.

We aimed to evaluate the pretreatment serum IP-10 levels as well as the IL-28B rs12979860 C/T polymorphisms as a predictive biomarkers for response to viral hepatitis C therapy in Egypt. Among these patients, 59 (72%) were SVRs and 23 (28%) were NRs.

It was reported that IP-10 is attractive as potential marker of treatment outcome due to its role as a regulator of immunity and inflammation in HCV infection. High IP-10 level may reduce the response of CXCR3 expressing T cells and have a negative effect on the outcome of treatment, while patients with low IP-10 levels can restore the response of CXCR3 expressing T cells assisting in control of the HCV [21]. Also, another study reported that responders have a lower baseline immune system activation before treatment, which becomes markedly induced in response to treatment by interferon [9].

In this study, patients with SVR had a significantly lower serum pretreatment IP-10 level, compared to NRs (318.18±79.52 vs. 732.57±235.67) and this is consistent with the other previous published studies [11,13,22].

ROC curve analysis showed a potentially useful IP-10 cutoff level in predicting the likelihood achieving SVR among our cohort. The pretreatment IP-10 level with the best sensitivity and specificity for identifying SVR was 499.02 pg/ml as all the responders in our cohort had a basal serum IP-10 ≤ 499.02 pg/ml (100% specificity) while 82.6% of the NRs had a basal serum IP-10 greater than 499.02 pg/ml. Al-Ashgar et al. (2013) [22] reported that a pretreatment threshold IP-10 level with the best compromise sensitivity and specificity to identify non-SVR was 359 pg/ml. A percentage of 81.8% of NRs were identified by IP-10 levels greater than 359 pg/ml and 45.2% of SVRs had IP-10 levels up to 359 pg/ml.

Lagging et al. (2006) [23] reported that in difficult-to-treat patients infected with HCV-1, a baseline IP-10 level more than 600 pg/ml was highly suggestive of an unfavorable treatment outcome. Moreover, a cutoff level of less than 150 pg/ml showed a high specificity (82–93%), but a low sensitivity (25–36%), and high PPV (50–76%) for identifying patients with a likelihood of SVR.

In the current study, the impact of IL-28B SNP rs12979860 on the response to treatment was also evaluated. Of the 82 patients genotyped for IL-28B SNP rs12979860, 70.7% were CC, 20.7% were CT, 8.5% were TT genotype.

We found that there was a statistically significant association between IL28B SNP rs12979860 genotype and the treatment response. The response rates were 86.2%, 52.9%, 0.0% for genotype CC, CT, and TT respectively. So we can say that carriage of a C allele at the IL-28B SNP rs12979860 is favorably associated with treatment

response. These results are generally consistent with the previously published studies on HCV genotype 4 [24,25], and with the previous studies reported on genotype 1 [14,26].

The IL28B gene encodes the protein interferon-λ-3 which is a type III IFN induced by viral infections [27]. While the mechanism behind the association of IL28B genotype and HCV clearance has not been yet elucidated, the innate immune response modulation is likely playing a role in the control of HCV infection. Further research on the mechanism by which genetic variations near the IL28B gene modulate the innate immune responses via IFN-λ are ongoing [13].

We also found a statistically significant lower baseline serum IP-10 levels in homozygous carriers of the favorable CC at rs12979860 and the risk allele T was found to be statistically associated with elevation of baseline serum IP-10 level (344.2±121.8 pg/ml for CC genotype vs. 576.02±283.21 and 838.01±240.86 for CT and TT respectively). These findings were in agreement with previous studies [10,28]. This is in line with the fact that there is an elevated baseline induction of interferon-stimulated genes (ISGs) among risk allele carriers [10].

We assessed whether other baseline parameters, in addition to IL28B SNP rs12979860 genotype and baseline serum IP-10 level, could significantly improve the prediction of SVR. In this study, patients with SVR were younger and had lower BMI. These findings are in agreement with previous studies that reported that older age and high BMI are negative predictors of response [29,30].

Baseline IP-10 level was found to be significantly positively correlated with age in this study. Since Lower baseline IP-10 level is associated with higher rate of SVR, this may explain why higher SVR rate is observed in younger patients. Moreover, Although age is an important factor for the outcome of treatment, it should be evaluated on a case-by-case basis according to the level of activity of the patient , life style [31] and age of infection (duration of infection) rather than age of patient since older patients develop a higher rate of liver fibrosis [32].

BMI was found to correlate with the degree of steatosis present in hepatitis C [29]. Steatosis results in elevation of the lipid deposits within the cells that may cause decrease of the contact area between the drugs and the hepatocytes containing the virus, resulting in functional disturbance that cause a reduction in the efficacy of the antiviral drug [33]. Moreover, the degree of steatosis was reported to correlate with the fibrosis severity [34]. In addition, in this study we found that there is a statistically highly significant positive correlation between IP-10 level and BMI which may explain also why high BMI is a negative response predictor.

There wasn't a significant difference in the baseline HCV RNA levels between SVR and the non-responders and this is consistent with the published studies of Saludes et al. (2010) [35] and Abdel-Rahman et al. (2013) [36]. However, these results differ from other previous studies which showed the significant association of low pretreatment viral load with SVR [37,38,39].

Therefore, our results did not confirm the importance of baseline HCV RNA levels for achieving SVR and this is also in line with results of Antonov et al. (2011) [40] which very clearly showed the advance of viral response during peg-IFN/ribavirin dual therapy above baseline viral and host predictive factors to predict SVR; early virological response is highly predictive of SVR. Also their finding of elevated baseline induction of interferon-stimulated genes (ISGs) among the NRs may explain the results of this study that the NRs had a lower baseline viral load than that observed in responders (although not reaching a statistically significant value which may be caused by small sample size).

Among the host-related factors evaluated in this study are the baseline ALT and AST levels which were significantly lower in patients with SVR . Zekri et al. (2013) reported that there was a statistically significant reduction in AST level in responders but ALT level didn't differ significantly between responders and non responders [41]. However, Saludes et al. (2010) reported that baseline ALT level was significantly higher in responder patients [35] .

Lower baseline ALT and AST levels observed in responders of this study may be correlated with the decreased serum IP-10 levels. This is consistent with the published study that revealed that elevated IP-10 level was positively correlated with liver damage (as indicated by high liver fibrosis score and liver enzymes level) [23]. Although the correlation of serum IP-10 level with AST and ALT levels in this study did not reach significant levels, this may be attributed to small sample size.

Our results showed a statistically significant impact of necroinflammatory activity grade (A) and fibrosis stage (F) on treatment response as we found that patients with moderate fibrosis and mild activity (METAVIR score ; A1F2) had a higher SVR rates (86.3% of A1F2 patients were responders while 13.7% were non responders). This finding is in accordance with the previously reported finding of poor SVR in patients with advanced liver necroinflammatory activity and fibrosis [39,42]. This may be because the hepatic inflammation severity is a major factor driving the chronic hepatitis C progression to cirrhosis [43].

The significant positive correlation found between serum IP-10 levels and fibrosis stage and the necroinflammatory activity in this study, supports the fact that a higher concentration of serum IP-10 may recruit more CD4+ T cells to the liver, inducing more vigorous immune reactions [44].

In agreement with Hoofnagle et al. (2009) [45] and in contrary to Saludes et al., (2010) [35] we found that male gender was significantly associated with treatment failure (25.4% of SVR were male while 75.9% were female). The disparate rates of viral clearance and response to therapy between HCV-infected males and females are still considered an unresolved mystery. However, it was found that testosterone enhanced the hepatic scavenger receptors expression and the tight junction protein claudin expression important for cellular entry of the HCV [46], while estrogen suppressed the hepatic scavenger receptors expression [47]. The importance of the testosterone in enhancing HCV entry into the host cells may explain the gender bias toward female in SVRs.

As regards to patients co-infected with schistosomiasis in the current study, no significant difference in treatment response between them and patients negative for Anti-schistosomal antibodies was found. This is consistent with the published study of Derbala et al. (2006) [32]. This could be explained by the observation of El Rafei et al. (2004) [48] who reported that patients infected with HCV genotype 4 can mount both CD4+ and CD8+ T cell-mediated HCV-specific responses, despite the presence of concomitant schistosomiasis.

CONCLUSION

Baseline serum IP-10 level is significantly associated with IL28B SNP rs12979860, and augments the predictiveness of the final treatment outcome. Therefore, both pre-treatment assessment of IL28B SNP rs12979860, and the measurement of baseline serum IP-10 level may provide helpful prognostic information prior to starting peg-INF/ RBV dual antiviral therapy.

REFERENCES

1.Kao J.H. and Chen, D.S. (2000) "Transmission of hepatitis C virus in Asia: past and present perspectives", J Gastroenterol Hepatol, 15(1) E91-E96.

2.El Makhzangy, H., Esmat, G., Said, M., ElRaziky, M., Shouman, S., Refai, R., Rekacewicz, C., Gad, R., Vignier, N., Abdel-Hamid, M., Zalata, Kh., Bedossa, P., Pol, S., Fontanet, A., and Mohamed, M. (2009) "Response to Pegylated Interferon Alfa-2a and Ribavirin in Chronic Hepatitis C Genotype 4", Journal of Medical Virology, 81 1576–1583.

3. Kamal, S.M. and Nasser I.A. (2008) Hepatitis C genotype 4: What we know and what we don't yet
Know, Hepatology, 47 1371-1383.

4. Strader, D.B., Wright, T., Thomas, D.L. and Seeff, L.B. (2004) "Diagnosis, management, and treatment of hepatitis C", Hepatology, 39 1147-1171.

5.Bochud, P.Y., Bibert, S., Negro, F., Haagmans, B., Soulier, A., Ferrari, C., Missale, G., Zeuzem, S., Pawlotsky, J.M., Schalm, S., Hellstrand, K., Neumann, A.U. and Lagging, M. (2011) "IL28B polymorphisms predict reduction of HCV RNA from the first day of therapy in chronic hepatitis C, Journal of Hepatology, 55(5) 980-988.

6.Lagging, M., Wejstal, R., Uhnoo, I., Gerden, B., Fischler, B. and Friman, S. (2009) " Treatment of hepatitis C virus infection: updated Swedish Consensus recommendations", Scand J Infect Dis, 41 389- 402.

7.Fried, M.W. (2002) "Side effects of therapy of hepatitis C", Hepatology, 36 S237-S244.

8.Hsu, C.S., Liu, C.H., Liu, C.J., Chen, C.L., Lai, M.Y., Chen, P.J., Chen, D.S. and Kao, J.H. (2009) "Factors affecting early viral load decline of Asian chronic hepatitis C patients receiving pegylated interferon plus ribavirin therapy", Antiviral therapy, 14(1) 45-54.

9. Sarasin-Filipowicz, M., Oakeley, E.J., Duong, F.H., Christen, V., Terracciano, L. and ilipowicz, W. (2008) "Interferon signaling and treatment outcome in chronic hepatitis C", Proc Natl Acad Sci USA, 105 7034–7039.

10. Lagging, M., Askarieh, G., Negro, F., Bibert, S. and So¨ derholm, J. (2011) " Response Prediction in Chronic Hepatitis C by Assessment of IP-10 and IL28B-Related Single Nucleotide Polymorphisms", PLoS ONE, 6(2) e17232.

11. Romero, A.I., Lagging, M., Westin, J., Dhillon, A.P., Dustin, L.B. (2006) "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, 194 895–903.

12.Zeremski, M., Petrovic, L.M. and Talal, A.H. (2007 b) "The role of chemokines as inflammatory mediators in chronic hepatitis C virus infection", J Viral Hepat., 14 675–687.

13.Darling, J., Aerssens, J., Fanning, G., McHutchison, J., Goldstein, D., Thompson, A., Shianna, K., Afdhal, N., Hudson, M., Howell, C., Talloen, W., Bollekens, J., De Wit, M., Scholliers, A., and Fried, M. (2011) " Quantitation of Pretreatment Serum IP-10 Improves the Predictive Value of an IL28B Gene Polymorphism for Hepatitis C Treatment Response", Hepatology, 53(1) 14–22.

14. Rauch, A., Kutalik, Z., Descombes, P., Cai, T., Di Iulio, J. and Mueller, T. (2010) " Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study", Gastroenterology, 138(4) 1338-45, 45 e1-7.

15. Tanaka, Y., Nishida, N., Sugiyama, M., Kurosaki, M. and Matsuura, K. (2009) "Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C", Nat Genet, 41 1105–1109.

16. Thomas, D.L., Thio, C.L., Martin, M.P., Qi, Y., Ge, D. and O'hUigin, C. (2009) " Genetic variation in IL28B and spontaneous clearance of hepatitis C virus", Nature, 461 798–801.

17.Suppiah, V., Moldovan, M., Ahlenstiel, G., Berg, T., Weltman, M. (2009) "IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy", Nat Genet, 41 1100–1104.

18. Egyptian national control strategy for viral hepatitis, 2008-2012. http://www.hepnile.org/index.php/national-strategy-document

19. Bhaskaran, K., Douglas, I., Forbes, H., dos-Santos-Silva I., Leon, D.A. and Smeeth,L. (2014) Body-mass index and risk of 22 specific cancers: A population-based cohort study of 5•24 million UK adults, Lancet, 384 755-765.

20. European Association for the Study of the Liver (2011) "EASL Clinical Practice Guidelines: management of hepatitis C virus infection", J. Hepatol., 55(2) 245–264.

21. Larrubia, J.R., Calvino, M., Benito, S., Sanz-de-Villalobos, E., Perna, C., Pérez-Hornedo, J., González-Mateos, F., García-Garzón, S., Bienvenido, A. and Parra, T. (2007) "The role of CCR5/CXCR3 expressing CD8+ cells in liver damage and viral control during persistent hepatitis C virus infection", J Hepatol, 47 632-641.

22. Al-Ashgara, H., Khana, M., Helmy, A., Al-Thawadi, S., Al-Ahdal, M., Khalaf, N., Al-Qahtani, A. and Sanai, F. (2013) " Relationship of interferon-c-inducible protein-10 kDa with viral response in patients with various heterogeneities of hepatitis C virus genotype-4", European Journal of Gastroenterology & Hepatology, 25 404–410.

23. Lagging, M., Romero, A.I., Westin, J., Norkrans, G., Dhillon, AP. and Pawlotsky, J.M. (2006) "IP-10 predicts viral response and therapeutic outcome in difficult-to-treat patients with HCV genotype 1 infection", Hepatology, 44 1617–1625.

24. Asselah, T., De Muynck, S., Broet, P., Masliah-Planchon, J., Blanluet, M. and Bieche, I. (2012) " IL28B polymorphism is associated with treatment response in patients with genotype 4 chronic hepatitis C", J Hepatol, 56(3) 527-32.

25. Khairy, M., Fouad, R., Mabrouk, M., El-Akel, W., Awad, A.B. , Salama, R., Elnegouly, M. and Shaker, O. (2013) " 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", Hepatitis Monthly, 13(7) e10509.

26. Ciesla, A., Bociaga-Jasik, M., Sobczyk-Krupiarz, I., Glowacki, M.K., Owczarek, D. and Cibor, D.(2012) " IL28B polymorphism as a predictor of antiviral response in chronic hepatitis C", World J Gastroenterol., 18(35) 4892-7.

27. Sheppard, P., Kindsvogel, W., Xu, W., Henderson, K., Schlutsmeyer, S., Whitmore, T.E. and Kuestner, R. (2003) "IL-28, IL-29 and their class II cytokine receptor IL-28R" Nat Immunol., 4 63–68.

28. Fattovich, G., Covolo, L., Bibert, S., Askarieh, G., Lagging, M., Clément, S., Malerba, G., Pasino, M., Guido, M., Puoti, M., Gaeta, G.B., Santantonio, T., Raimondo, G., Bruno, R., Bochud, P.Y., Donato, F., Negro, F. and ITAHEC Study Group (2011) " IL28B polymorphisms, IP-10 and viral load predict virological response to therapy in chronic hepatitis C", Aliment Pharmacol Ther, 33(10) 1162–1172.

29. Bressler, B.L., Guindi, M., Tomlinson, G. and Heathcote, J. (2003) " High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C", Hepatology, 38 639-644.

30. Durante-Mangoni, E., Zampino, R. and Portella, G. (2009) "Correlates and prognostic value of the first-phase hepatitis C virus RNA kinetics during treatment", ClinInfect Dis, 10 43-49.

31. Esmat, G., El Kassas, M., Hassany, M., Gamil, M.E. and El Raziky, M. (2013) " How to optimize HCV therapy in genotype 4 patients", Liver Int., 33(1): 41-45.

32. Derbala, M.F., Al Kaabi, S.R., El Dweik, N.Z., Pasic, F., Butt, M.T., Yakoob, R., Al-Marri, A., Amer, A.M., Morad, N. and Bener, A. (2006) " Treatment of hepatitis C virus genotype 4 with peginterferon alfa-2a: Impact of bilharziasis and fi brosis stage", World J Gastroenterol, 12(35) 5692-5698.

33. Giannini, E., Ceppa, P. and Testa, R. (2001) " Steatosis in chronic hepatitis C: can weight reduction improve therapeutic efficacy?", J Hepatol, 35 432-433.

34. Hourigan, L.F., Macdonald, G.A., Purdie, D., Whitehall, V.H., Shorthouse, C. and Clouston, A. (1999) "Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis", Hepatology, 29(4) 1215-1219.

35. Saludes, V., Bracho, M.A., Valero, O., Ardèvol, M., Planas, R., González-Candelas, F., Ausina, V. and Martró, E. (2010) " Baseline prediction of combination therapy

outcome in hepatitis C virus 1b infected patients by discriminant analysis using viral and host factors", PLoS ONE, 5(11) e14132.

36. Abdel-Rahman, M., El-Sayed, M., El Raziky, M., Elsharkawy, A., El-Akel, W., Ghoneim, H., Khattab, H., and Esmat, G. (2013) " Coinfection with hepatitis C virus and schistosomiasis: Fibrosis and treatment response", World J Gastroenterol., 19(17) 2691–2696.

37. Zeuzem, S., Buti, M. and Ferenci, P. (2006) "Efficacy of 24 weeks treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia", J Hepatol, 44 97-103.

38. Moucari, R,, Asselah, T., Cazals-Hatem, D., Voitot, H., Boye, N., Ripault, M.P., Paradis, V., Vidaud, M., Valla, D. and Marcellin, P.(2008) "Insulin resistance in chronic hepatitis C: association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis", Gastroenterology, 134 416-423.

39. Faisal1, A., Zytoon, A., Gad Allah, A. and Dawood, A. (2013) " Predictors of Early Virological Response of Viral Hepatitis C to Combination Therapy with Pegylated Interferon Plus Ribavirin", American Journal of Clinical Medicine Research, 1(4) 54-60.

40. Antonov, K., Jelev, D., Ivanova, A. and Krastev, Z. (2011) " predictors of sustained virological response (svr) to pegylated interferon alpha (peg-ifn α) and ribavirin (rbv) in patients with chronic hepatitis c infected with genotype 1", journal of imab, 17(1) 197-199.

41. Zekri, A.R., Bahnassy, A.A., Mohamed, W.S., Alam El-Din, H.M., Shousha, H.I., Zayed, N., Eldahshan, D.H. and Abdel-Aziz, A.O. (2013) " Dynamic interplay between CXCL levels in chronic hepatitis C patients treated by interferon", Virology Journal, 10 218.

42. Balart, L., Lee, S.S., Everson, G.T., Reindollar, R.W., Shiffman, M.L. and Minuk, G.Y. (2008) "Histological benefits of virological response to peginterferon alfa-2a monotherapy in patients with hepatitis C and advanced fibrosis or compensated cirrhosis", Aliment Pharmacol Ther , 27 542-551.

43. Pockros, P.J., Hamzeh, F.M., Martin, P., Lentz, E., Zhou, X. and Govindarajan, S. (2010) " Histologic Outcomes in Hepatitis C Infected Patients with Varying Degrees of Virologic Response to Interferon-Based Treatments", Hepatology, 52(4) 1193-1200.

44. You, C.R., Park, S., Jeong, S.W., Woo, H.Y., Bae, S.H., Choi, J.Y., Sung, Y.C., and Yoon, S.K. (2011) " Serum IP-10 Levels Correlate with the Severity of Liver Histopathology in Patients Infected with Genotype-1 HCV",Gut Liver, 5(4) 506–512.

45. Hoofnagle, J.H., Wahed, A.S., Brown JR, R.S., Howell CD and Belle SH (2009) " Early changes in hepatitis C virus (HCV) levels in response to peginterferon and ribavirin treatment in patients with chronic HCV genotype 1 infection",J Infect Dis, 199 1112–1120.

46. Langer, C., Gansz, B. and Goepfert, C. (2002) "Testosterone up-regulates scavenger receptor BI and stimulates cholesterol efflux from macrophages", Biochem. Biophys. Res. Commun., 296(5) 1051–1057.

47. Srivastava, R.A. (2003) "Scavenger receptor class B type I expression in murine brain and regulation by estrogen and dietary cholesterol", J. Neurol. Sci., 210(1–2) 11–18.

48. Elrefaei, M., El-sheikh, N., Kamal, K. and Cao, H. (2004) " Analysis of T cell responses against hepatitis C virus genotype 4 in Egypt", J Hepatol, 40 313-318.