

Hepatitis C Viral Kinetic Changes in a Retrospective Cohort Study of Chronic Hepatitis C Virus Egyptian Patients on Pegylated Interferon and Ribavirin Therapy

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The aim of this study was to determine the relative importance of the kinetics of antiviral response compared to baseline host and virological factors for predicting treatment outcome. A retrospective analysis of 285 chronic hepatitis C virus (HCV) patients, encompassing genotypes 4 treated with peginterferon alpha-2a and ribavirin, was performed. Baseline characteristics were compared across HCV genotypes and pretreatment factors associated with rapid virological response (RVR) were identified. The relative significance of RVR compared to other baseline factors for predicting sustained virological response was analyzed by multiple logistic regression analysis. Ninety-seven percent of the patients harbored HCV genotype 4a patients. The positive predictive value (PPV) of RVR for end-of-treatment response (ETR) was 88% and of early virological response (EVR) was 85%, which means that achievement of both RVR and EVR is a good positive predictive factor of response. The negative predictive value (NPV) of RVR for ETR was low and equals 26.77%, which means that approximately two-thirds of patients were able to achieve ETR despite not experiencing RVR, which means RVR is a bad negative predictive factor of response. The NPV of EVR for ETR was high and equals 90%, which means that only 10% of patients were able to achieve an ETR despite not experiencing EVR, which explains that EVR is a very good negative predictive factor of response. In univariate logistic regression analysis, which included the following: female gender, alanine aminotransferase, aspartate transaminase, α -fetoprotein, baseline HCV-RNA levels, grade of activity, stage of fibrosis, and positive HCV-RNA, by polymerase chain reaction at week 4, none of the previous factors was a significant independent factor of failure of response to treatment. The current study demonstrated that a viremia at week 4 has a good PPV, but it has a very low NPV. The NPV of EVR was more robust for ETR (90%). EVR is regarded as a robust indicator of treatment outcome, and a 12-week stopping rule for patients is strongly evident.

Introduction

EGYPT HAS THE uppermost prevalence rate of hepatitis C virus (HCV) in the world. It is been reported that nosocomial transmission has been, and probably still is, the most common route for new infections (Paez Jimenez and others 2010). The Egyptian Demographic Health Survey conducted in 2008 reported seroprevalence and viremia rates for individuals aged 15–59 years old as 14.7% and 9.8%, respectively (El-Zanaty and Way 2009). Including a population younger than 15 years in the same period made a decline in viremic prevalence at 12% (Razavi and others 2014). On the other hand, only two-thirds of the infected

population was viremic in the EDHS, resulting in an all age group viremic prevalence of 8.5% in 2008. Estimate of 2013 HCV-infected populations' viremic prevalence was 7.3%, the viremic prevalence dropped by 1.2%, and the actual number of cases decreased by 300,000 cases. However, the increase in population in the last 5 years was responsible for some of the drop in estimated HCV prevalence (Central Agency for Public Mobilization and Statistics 2013).

The genotype distribution in Egypt is mainly genotype 4 (HCV-G4), which is responsible for more than 90% of the infections, with mainly HCV-G1 (Varghese and others 2009).

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HCV-*G4* has been considered “difficult to treat” with pegylated interferon (PEG-IFN) and ribavirin (RBV), with sustained virological response (SVR) rates better than HCV-*G1* and worse than HCV-*G2* and -*G3* (Irshad and others 2010). The recent approval of sofosbuvir (SOF) for treatment of HCV-*G4* (Abdel-Razek and Waked 2015) promises significant improvement in the outcome of therapy.

The Ministry of Health in Egypt has embarked on a national treatment program for patients with chronic HCV infection since 2006, where all eligible patients are treated with PEG-RBV, with almost 100% of the patients receiving therapy for free. Annually, 40,000–50,000 patients have been treated, and by 2013, 350,000 patients have received therapy in this program (Doss and others 2008). SVR rates for patients treated with the original PEG-IFN alpha-2a and alpha-2b are 54%–59% (El Raziky and others 2013; Esmat and others 2014), and response rates to a locally produced biosimilar PEG-IFN were reported at 52% (Taha and others 2010; Health Insurance Organization Higher Committee for Liver Disease: data on file. 2013, unpublished data). Predictors of response of HCV-*G4*-infected patients to therapy with PEG-RBV include viral load, fibrosis score, and ethnic origin (Gad and others 2008; El Makhzangy and others 2009; Moucari and others 2009). An overall better response was observed in patients infected with the *4a* subtype (60% versus 35% for *non-4a* subtypes) and SVR rates were higher in patients infected in Egypt, compared with those infected in France or Africa (54.9%, 40.3%, and 32.4%, respectively, $P < 0.05$) (Roulot and others 2007).

A major predictor of response to PEG-RBV therapy in patients with HCV-*G4* is the *IL-28B* genotype (Asselah and others 2012; De Nicola and others 2012; Stättermayer and others 2014). The favorable *CC* phenotype is found in 20%–30% of Egyptian patients with chronic hepatitis C (Antaki and others 2013; El Awady and others 2013; Ragheb and others 2014). Those other reports did not find this relation. Another predictor of response to PEG-RBV therapy was found to be insulin resistance, where high HOMA-IR score was found to impair response rates to PEG-RBV therapy in HCV-*G4* patients. Some reports during treatment with PEG-RBV, patients with HCV-*G4* who achieve a rapid virological response (RVR, HCV-RNA negative at 4 weeks of starting therapy) respond much better than those who do not (Khatab and others 2010), and in these patients, several studies have shown that response rates are equally high if they are treated for a total of 24 or 48 weeks (Ferenci and others 2008; El Khayat and others 2012; Marcellin and others 2012). An expert panel (Khatab and others 2011) recommended shortening therapy to 24 weeks for those who achieve an RVR and do not have unfavorable predictors of response (high viral load, advanced fibrosis, and insulin resistance).

They also recommended extending therapy for 72 weeks for slow responders who have >2 log decrease in HCV-RNA by 12 weeks and become RNA negative at 24 weeks. The national program in Egypt, however, treats all patients for 48 weeks.

Attainment of an RVR, defined as undetectable HCV-RNA at week 4 during treatment with PEG-IFN and ribavirin, is highly predictive of SVR. The aim of this study was to determine the relative importance of the viral kinetics changes at week 4 compared to baseline host and virological factors for predicting treatment outcome.

Methodology and Work Plan

Study design and patient sample

This is a retrospective study conducted on 285 Egyptian chronic hepatitis C-infected patients who attended El-Fatemia Hospital and were naive to treatment. Patients underwent treatment by PEG-IFN and ribavirin and were followed during treatment at week 12 [early virological response (EVR)], week 24, and at week 48 [end-of-treatment response (ETR)]. Patients who were treated with PEG-IFN alpha plus RBV were analyzed for quantitative HCV-RNA load at week 4 of treatment after taking written informed consent from the patients. Patients were included and treated according to the Egyptian guidelines for HCV treatment. Sample size was estimated using a power calculation program to be at least 200 patients to achieve a confidence level of 85% and re-estimated to be at least 150 to achieve a confidence level of 78% (Raosoft program; www.raosoft.com/samplesize.html).

Study medication

All patients were treated with a weight-based 1.5 µg/kg weekly dose of subcutaneous PEG-IFN alpha-2b or 180 µg weekly dose of subcutaneous PEG-IFN alpha-2a. In combination with PEG-IFN alpha-2a or b, RBV was given orally at a daily dose of 800–1,400 mg based on bodyweight (800 mg for patients weighing <65 kg, 1,000 mg for those weighing 65–85 kg, 1,200 mg for those weighing 85–105 kg, and 1,400 mg for those weighing >105 kg). The length of the combined treatment was 48 weeks.

Data collection

Patients’ data were thoroughly recorded in medical records, ensuring security of the data collected and adequate patients’ confidentiality.

From the stored information in the medical database of the hospital, the following was used: full epidemiological and clinical data, the results of the treatment, the results of quantitative polymerase chain reaction (PCR) at week 12, the results at the end of treatment, and the results of abdominal ultrasonography and liver function, which were done at regular visits of the patients according to the designed protocol for treatment.

Sample preparation, collection, and storage

Sample preparation (serum): a 5 mL blood sample was collected from eligible patients into sterile tubes, allowed to clot at room temperature for 30 min, and then centrifuged. Sera were separated, aliquoted, and stored at -80°C until used.

Determination of HCV-RNA level

We retrospectively determined serum HCV-RNA level by TaqMan HCV assay (Applied Biosystems, Foster City, CA) for each patient (at week 4); these samples were stored at -80°C to ensure RNA validity. The TaqMan has a lower limit of quantitation of 15 IU/mL and an outer limit of quantitation of 6.9×10^7 IU/mL. The quantitative reverse transcription-PCR (QRT-PCR) protocol of HCV was conducted using TaqMan assay as follows:

RNA extractions from 285 stored frozen samples were performed using the QIA amp viral RNA Mini kit (Qiagen,

Hilden, Germany) according to the manufacturer's instructions supplied with the kit. Extracted RNA was measured and quantitated with a UV spectrophotometer at 260–280 wave length to assess successful and purified RNA quality. The HCV standards and their serial dilutions prepared using seronegative plasma were used to generate calibration curves and evaluate the 95% detection limits. QRT-PCR was done following RNA extraction. All reagents were obtained from Applied Biosystems. The AgPath-ID™ One-Step RT-PCR kit includes an enzyme mixture, buffer, and detection enhancer for one-step QRT-PCR. RT-PCR one step master mix was performed in a final volume of 25 μ L reaction volume.

HCV genotyping assessment

Genotyping was done using conventional multiplex RT-PCR (Thermo Scientific Verso 1-Step RT-PCR Ready Mix Kit, Literature Code: AB-1454-LD-v8-0411).

Genotyping methodology included c DNA synthesis and first round PCR by first strand PCR primers followed by second round PCR using specific primers mix for each HCV genotyping.

Statistical analyses

Patients were categorized into rapid responders (patients who achieved negative PCR at week 4 and 48), slow responders (patients who did not achieve negative PCR in week 4, but achieved negative PCR at week 48), and nonresponders (patients who did not achieve negative PCR at any interval).

Comparison between responders (rapid and slow) and nonresponders was done using Student's *t*-test or analysis of variance (ANOVA) for quantitative variables and chi-square of Fischer's exact test for qualitative variables.

Positive predictive value (PPV) of EVR for ETR as an example was calculated by the following equation:

$$\text{PPV of EVR for ETR} = \frac{\text{no of patients with EVR and ETR}}{\text{no of patients with EVR}} \times 100$$

Negative predictive value (NPV) of EVR for ETR as an example was calculated by the following equation:

$$\text{NPV of EVR for ETR} = \frac{\text{no of patients without EVR and ETR}}{\text{no of patients without EVR}} \times 100$$

Receiver operator characteristic (ROC) curve was constructed to assess the baseline viral load level as predictor for an RVR area under the curve (AUC), and the best cutoff value was used to determine which variables better predict response to treatment at week 4, 12, and 48. AUC was considered significant if its *P* value <0.05. ROC analysis with nonparametric (Spearman) correlation was done to correlate baseline viral load with week 4 viremia.

In univariate and multivariate binary logistic regression analyses, failure of response at end of treatment (week 48) was considered the dependent variable. In all tests, *P* value was considered significant if <0.05.

The main outcome was rapid viral response to treatment at week 4. The analyses included all patients (*n*=285, 39 rapid viral responders, 186 slow viral responders, and 60 nonresponders). The descriptive statistics were presented as numbers and proportions for categorical variables. For continuous variables, either mean and standard deviation or median and interquartile range were reported. Variables not normally distributed, such as viral load, were log transformed for analysis, and geometric means were reported.

Baseline characteristics were compared across the treatment groups (rapid viral responders versus slow viral responders and nonresponders). The association between these factors and response to treatment was assessed first using univariate analysis 2 \times 2 tables. Chi-square test was used for categorical variables. For continuous variables, either independent samples *t*-tests (for parametric data) or Mann-Whitney test (for nonparametric data) were used. Crude odds ratio (OR) were calculated using either chi-square tests or univariate logistic regression. Two-sided *P* values and 95% confidence intervals were reported for all tests. *P* values <0.05 were considered to be statistically significant. Pearson (for normally distributed data) and Spearman (for nonparametric data) correlation coefficients between key variables were estimated.

To determine the predictors of response to treatment, multivariate analysis for continuous variables was performed using ANOVA. For categorical variables, multivariate logistic regression was used with adjustment for possible confounders, such as age and gender. All factors associated with response to treatment with a significance level of <0.25 were entered in the model. Only variables with *P* value <0.05 remained. Adjusted ORs, 2-sided *P* values, and 95% confidence intervals were calculated.

All analyses were performed using SPSS 18.0 statistical software (SPSS for Windows, Rel. 11.0.1. 2001; SPSS, Inc., Chicago, IL).

Results

Two hundred eighty-five HCV-RNA-extracted samples were subjected to conventional one-step RT-PCR primers mix to update, assess, and evaluate HCV genotyping and most samples (276 patients, 96.8%) were positive for HCV genotype 4a, 6 patients (1.76%) were positive for HCV genotype 3a, and 3 patients (1.32%) were positive for HCV genotype 1b.

Patients were followed up after starting treatment at week 4 (RVR), 12 (EVR), 24, and 48 (ETR) and classified into 3 groups as shown in Figs. 1 and 2 and Table 1.

On comparing patients who were adherent to therapy, the following Table 2 shows comparison of demographic features among 3 groups. We observed the following:

Regarding age, gender, and body mass index (BMI), no significant statistical difference was observed between rapid responders, slow responders, and nonresponders.

There is no significant relation between gender, age, BMI, and RVR.

Baseline HCV-RNA level is significantly lower in the rapid responders group than the nonresponder (NR) group.

Lower baseline absolute neutrophil count (ANC) level is approaching significance in rapid responders group than the other 2 groups.

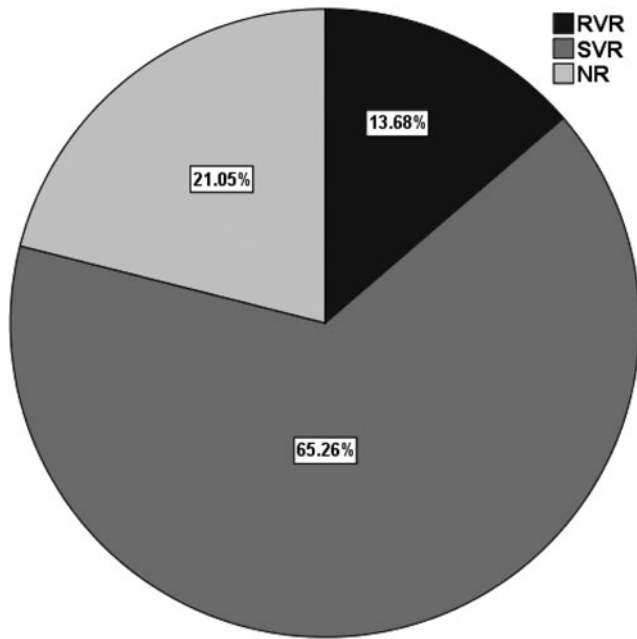


FIG. 1. Response to treatment groups (RVR/SVR/NR). NR, nonresponder; RVR, rapid virological response; SVR, sustained virological response.

Baseline thyroid stimulating hormone (TSH) is significantly higher in rapid responders group than the slow responders group. Regarding degree of activity and stage of fibrosis:

There is no significant statistical difference in the degree of hepatic fibrosis and the hyaluronic acid index (HAI) between the studied groups ($P=0.22$ and 0.18 , respectively).

None of the above parameters is significantly different in the RVR group than the non-RVR group.

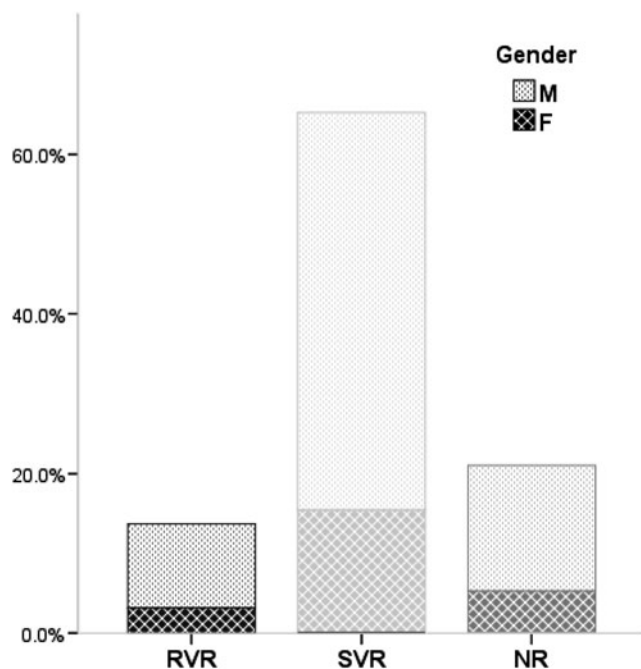


FIG. 2. Gender distribution among response groups.

TABLE 1. RESPONSE TO TREATMENT AND GENDER IN RELATION TO THE GRAND TOTAL

	Males, n (%)	Females, n (%)	Total
RVR	30 (10.5)	9 (3.2)	39 (13.7)
SVR	142 (49.8)	44 (15.4)	186 (65.3)
NR	45 (15.8)	15 (5.3)	60 (21.1)
Total	217 (76.1)	68 (23.9)	285

NR, nonresponder; RVR, rapid virological response; SVR, sustained virological response.

There is no significant agreement between results of HCV-RNA by qualitative PCR between week 4 and 12 in the studied patients. $P=0.63$ (not significant, NS) as shown in Table 3.

None of the above parameters is a significant independent factor associated with failure of response to treatment on the level of univariate analysis, so multivariate analysis was not done.

Correlation between baseline and week 4 HCV-RNA levels is shown in Table 4.

There is a significant positive association between both baseline and week 4 HCV-RNA levels.

Relation between RVR, EVR, and ETR:

$$PPV \text{ of RVR for ETR} = \frac{\text{no of patients with RVR and ETR}}{\text{no of patients with RVR}} \times 100$$

Therefore, PPV equals $22/25 \times 100 = 88\%$

NPV of RVR for ETR

$$NPV \text{ of RVR for ETR} = \frac{\text{no of patients didn't achieve RVR and ETR}}{\text{no of patients didn't achieve RVR}} \times 100 = 34/127 \times 100 = 26.77\%$$

Percentage of patients who did not achieve an RVR and achieved an ETR = $93/127 \times 100 = 73.22\%$

$$PPV \text{ of EVR for ETR} = \frac{\text{no of patients with EVR and ETR}}{\text{no of patients with EVR}} \times 100$$

Therefore, PPV equals $91/107 \times 100 = 85\%$

NPV of EVR for ETR

$$NPV \text{ of EVR for ETR} = \frac{\text{no of patients didn't achieve EVR and ETR}}{\text{no of patients didn't achieve EVR}} \times 100 = 18/20 \times 100 = 90\%$$

Percentage of patients who did not achieve an EVR and achieved ETR = $2/20 \times 100 = 10\%$

There is a significant difference between accuracy of PCR results at week 4 and 12 for prediction of ETR in favor of week 12 ($P < 0.01$).

On constructing ROC curve to assess predictive value of baseline HCV-RNA level in discrimination between the RVR group (group I) and non-RVR groups, it revealed the

TABLE 2. UNIVARIATE ANALYSIS COMPARING RAPID VIRAL RESPONDERS (N=39) TO SLOW VIRAL RESPONDERS (N=186) AND NONRESPONDERS (N=60)

Baseline characteristics	Rapid viral responders (n=39)	Slow viral responders (n=186)	Nonresponders (n=60)	P
Clinical data				
Male sex, n (%)	30 (76.9)	142 (76.3)	46 (75)	0.970
Mean age (SD), years	43.6 (7.8)	41.2 (9.8)	45.8 (10.8)	0.241
Age groups, n (%)				0.207
<35	3 (14.3)	17 (25.4)	4 (25)	
35-44	9 (42.9)	22 (32.8)	2 (12.5)	
45-54	8 (38.1)	23 (34.3)	6 (37.5)	
55+	1 (4.8)	5 (7.5)	4 (25)	
BMI	28 (3.5)	27.4 (3.8)	28.1 (4.3)	0.493
Biological data, mean (SD) serum level				
ALT, IU/L	56.7 (31.4)	47.2 (30.5)	45.2 (21.3)	0.170
AST, IU/L	47.1 (22.1)	44.1 (40.1)	38.8 (13.3)	0.515
Total bilirubin, mg/dL	0.77 (0.2)	0.75 (0.3)	0.83 (0.3)	0.235
Platelet count/mm ³	217.9 (65.9)	220.1 (68.4)	201 (52.8)	0.237
Hemoglobin, g/dL	14.6 (1.4)	14.1 (1.6)	13.9 (1.3)	0.150
α-Fetoprotein, ng/mL	4.2 (4.4)	5.2 (6)	6.9 (5.3)	0.100
Alkaline phosphatase, IU/L	113.3 (67.7)	94.7 (56.6)	105 (58.1)	0.216
Albumin, g/dL	4.3 (0.4)	4.3 (0.4)	4.3 (0.4)	0.387
Virological data				
HCV-RNA viral load, mean (SD), 10 ³ IU/L	2172.4 (1069)	3705.5 (2636)	608.8 (795.9)	0.709
HCV LogRNA viral load, mean (SD)	4.7 (1.2)	5.2 (1.2)	5.4 (0.8)	0.022
Liver histology				
Fibrosis score (Metavir score), n (%)				
Minimal fibrosis	19 (61.3)	89 (67.9)	28 (62.2)	0.670
Significant fibrosis	12 (38.7)	42 (32.1)	17 (37.8)	
Activity score				
Low activity	18 (58.1)	90 (69.8)	32 (72.7)	0.363
High activity	13 (41.9)	39 (30.2)	12 (27.3)	

This table contains mean and SD, n (%). Tests used are independent samples t-test and chi-square test. BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate transaminase; HCV, hepatitis C virus; SD, standard deviation.

following: AUC of 0.65 (P=0.01, significant) with best cutoff 5.23 log₁₀ IU/mL at which sensitivity, specificity, PPV, and NPV were 62%, 65%, 89%, and 26%, respectively (Fig. 3C).

On constructing ROC curve to assess predictive value of baseline HCV-RNA level in differentiation between rapid responders group (group I) and slow responders group, it revealed the following: AUC of 0.61 (P=0.15, NS) (Fig. 3D).

On constructing ROC curve to assess predictive value of baseline HCV-RNA level in differentiation between responders groups and nonresponder group revealed the following: AUC of 0.66 (P=0.018, significant) with best cutoff 5.5 log₁₀ IU/mL at which sensitivity, specificity, PPV, and NPV were 73%, 62%, 48%, and 45%, respectively (Fig. 3E).

Discussion

Because of the moderate efficacy of treatment, its long-term duration and the relative high incidence of side effects that might need extra cost for treatment, accurate prediction of response before initiation of therapy is critical (Kau and others 2008). Several previous studies have reported that patients who achieved an RVR had a high likelihood of achieving an SVR. However, there are relatively few patients infected with HCV genotype 4a who achieve an RVR.

TABLE 3. RELATIONSHIP BETWEEN RVR AND EVR

<i>EVR-RVR cross tabulation</i>				
<i>PCR at week 4</i>				
		-ve PCR	+ve PCR	Total
PCR at week 12				
-ve PCR	Count	22	107	129
	% Within EVR	17.1	82.9	100.0
	% Within RVR	88.0	84.3	84.9
+ve PCR	Count	3	20	23
	% Within EVR	13.0	87.0	100.0
	% Within RVR	12.0	15.7	15.1
Total	Count	25	127	152
	% Within EVR	16.4	83.6	100.0
	% Within RVR	100.0	100.0	100.0

Kappa for agreement 0.01, P=0.63 (NS).

EVR, early virological response; NS, not significant; PCR, polymerase chain reaction.

TABLE 4. CORRELATION BETWEEN BASELINE AND WEEK 4 HCV-RNA LEVELS

		Baseline HCV-RNA load
Spearman's correlation	PCR_W4	0.271 ^a
	Correlation coefficient	
	P	0.006

^aCorrelation is significant at the 0.01 level.

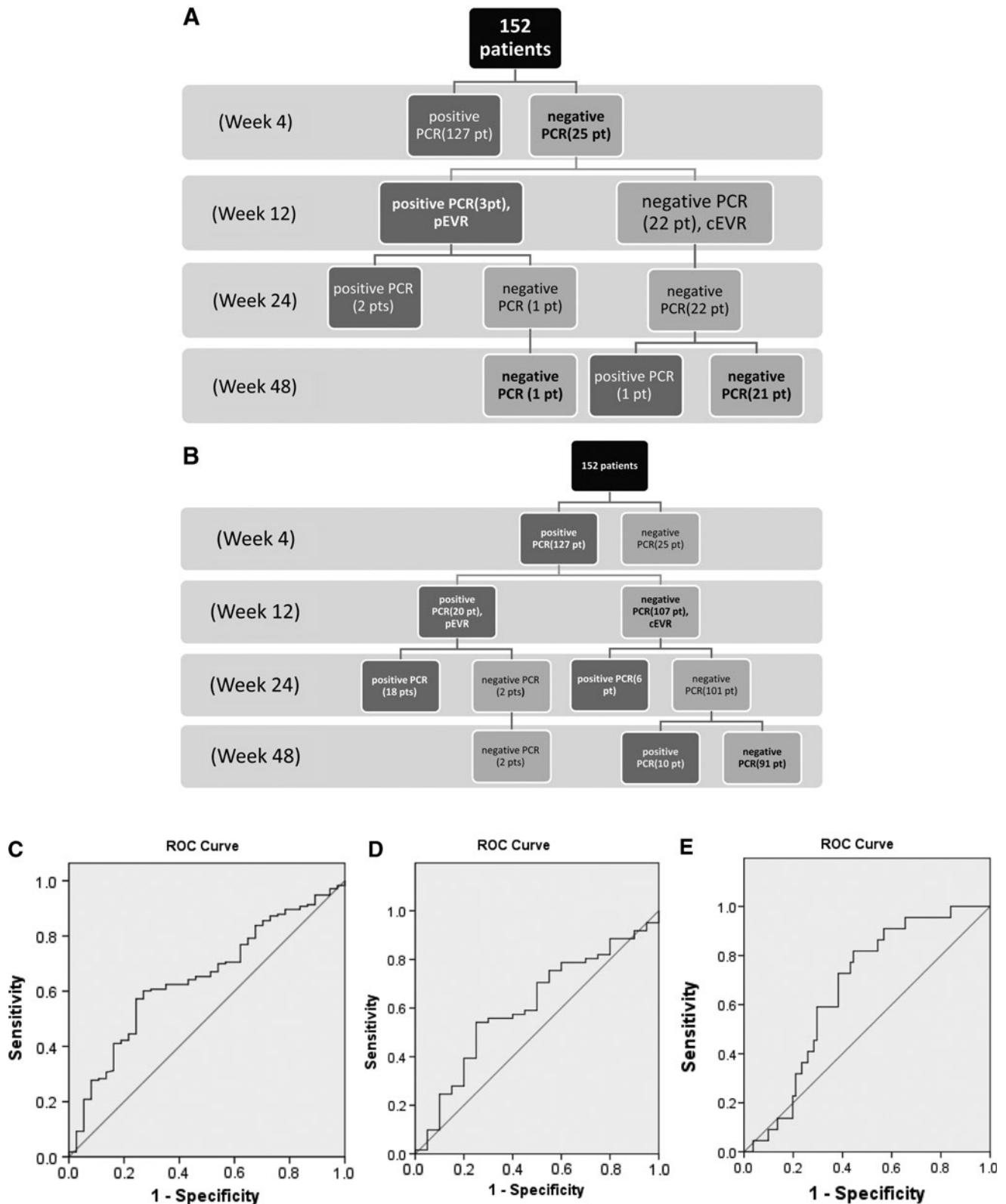


FIG. 3. (A) Flow diagram of negative patients for HCV-RNA at week 4. (B) Flow diagram of positive patients for HCV-RNA at week 4. (C) The ROC analysis for the prediction of rapid virologic response to combination therapy with PEG-IFN alpha-2a and RBV according to the baseline HCV-RNA level. (D) The ROC analysis for the prediction of rapid responders and slow responders to combination therapy with PEG-IFN alpha-2a and RBV according to the baseline HCV-RNA level. (E) The ROC curve analysis for the prediction of rapid, slow responders (group I and II), and nonresponder group (group III) to combination therapy with PEG-IFN alpha-2a and RBV according to the baseline HCV-RNA level. HCV, hepatitis C virus; PEG-IFN, pegylated interferon; RBV, ribavirin; ROC, receiver operating characteristics.

A considerable percentage of patients achieve an SVR even without an RVR. Therefore, an RVR has high specificity, but low sensitivity for predicting the ETR as well as an SVR (Poordad and others 2008; de Segadas-Soares and others 2009; Martinot-Peignoux and others 2009).

In this study, we evaluated the ability of an RVR to predict the likelihood of an ETR in Egyptian patients with chronic HCV, based on data from 152 patients with chronic hepatitis C naive to treatment, who completed a combination therapy with PEG-IFN and ribavirin and found that PPV of ETR for patients with an RVR was 88%, while NPV of RVR for ETR was 26.77%. In other words, 73.22% of HCV-infected patients were able to achieve an ETR despite not achieving an RVR, as shown in Figs. 3A and 3B.

This goes with a study done on HCV-4 by Taha and others (2010) who showed that PPV of ETR and SVR for patients with an RVR was 100% and 91.7%, respectively.

Also, our study goes with another study done by Raboisson and others (2008) who showed that PPV of SVR for patients with an RVR was 69% for HCV-1, 90% for HCV-3, and 83% for HCV-4.

However, our study disagrees with Raboisson and others (2008) in NPV of RVR for an SVR, which was 70% in HCV genotype 1 and 4 and 43% in genotype 3.

In this study, PPV of ETR in patients with an EVR was 85%, while NPV of EVR for ETR was 90%. In other words, only 10% of HCV-infected patients were able to achieve an ETR despite not achieving an EVR, while more than 73% of HCV-infected patients were able to achieve an ETR despite not achieving an RVR. Thus, the achievement of ETR in HCV-infected patients in our study was mainly driven by the achievement of an EVR as NPV of ETR for patients with an EVR was high (90%).

This goes with a study done on HCV-4 by Taha and others (2010) who showed that PPV of ETR and SVR in patients with an EVR was 87.1% and 67.74%, respectively.

When we correlated different demographic parameters with response to treatment, there was no statistically significant difference between rapid, slow, and nonresponders regarding age, gender, or BMI with *P* values of 0.69, 0.98, and 0.74, respectively.

Our results are similar to results of a study done by El Makhzangy and others (2009) on 95 Egyptian patients with HCV-4 who reported no impact of age (≤ 40 years, > 40 years), gender, or BMI on SVR.

Regarding virological and biochemical parameters, there is a significant correlation between the HCV-RNA level and response to treatment as the baseline HCV-RNA level is significantly lower in rapid responders group than the NR group, *P*=0.033.

This finding is in agreement with Shiffman and others (2007), who concluded that a low baseline viral load (600,000 IU/mL or less) was an independent predictor of SVR regardless of the genotype.

These results disagree with El Makhzangy and others (2009) who found that there was no impact of viral load ($\leq 600,000$, $> 600,000$ IU/mL) on the SVR.

In our study, alanine aminotransferase (ALT) is high in rapid responders and slow responders group versus the NR group with nonsignificant *P* value=0.697.

A study showed that baseline ALT levels were not associated with treatment response in the multilogistic regression analysis (Berg and others 2006).

In our study, the level of α -fetoprotein (AFP) is high in the NR group versus rapid responders and slow responders groups with nonsignificant *P* value=0.455. This disagrees with Males and others (2007) who showed that higher serum AFP was found to be independently and negatively associated with SVR in Egyptian patients with HCV genotype 4.

In this study, baseline TSH is significantly higher in rapid responders group than the slow responders group, *P*=0.039. Zantut-Wittmann and others (2011) concluded that HCV patients may develop central hypothyroidism either due to viral infection or during the interferon treatment. These patients presented 3.83 times more chance of not obtaining a sustained virological response.

In this study, the baseline ANC is significantly lower in rapid responders group than slow responders and nonresponders group, *P*=0.057; this disagrees with Oze and others (2013) who concluded that white blood cells (WBCs) count has no significant association with SVR through univariate logistic regression analysis, but on the other hand showed significant association with virological NR by univariate analysis. When evaluated by multivariate analysis, it showed no significant relation with NR.

Regarding histopathological parameters, there is no significant statistical difference in the degree of hepatic fibrosis and the HAI between the studied groups (*P*=0.22 and 0.18, respectively).

Our results disagree with El Makhzangy and others (2009) who found that patients with Metavir fibrosis score F1 or F2 had a significantly more frequent SVR compared with those with more advanced fibrosis F3/F4, 43/62 (69%) versus 15/33 (45%), *P*=0.02.

The relationship between baseline parameters in our studied 152 patients and RVR showed no significant statistical difference in the age, BMI, or gender between the studied groups.

Also, no significant statistical difference was shown between the RVR and non-RVR group regarding baseline biochemical parameters and viral load level.

In this study, we correlated baseline and week 4 HCV-RNA viral loads using spearman's correlation and found a significant association between both baseline and week 4 HCV-RNA levels.

This agrees with a study done by Fried and others (2011) on 1,383 patients with HCV genotypes 1–4 who found that low baseline HCV-RNA level is an independent predictor of RVR.

To identify suitable thresholds of baseline HCV-RNA for predicting virological response for all patients, ROC curves were calculated and specificity plus sensitivity were maximized.

In this study, after constructing an ROC curve to assess the predictive value of baseline HCV-RNA level in discrimination between RVR (group I) and non-RVR groups (group II and III), it revealed the following: AUC of 0.65 with a significant *P* value of 0.01, with best cutoff 5.23 log₁₀ IU/mL at which sensitivity, specificity, PPV, and NPV were 62%, 65%, 89%, and 26%, respectively.

Also, the ROC curve used to assess predictive value of baseline HCV-RNA level in differentiation between responders groups (group I and II) and nonresponder group (group III) revealed the following: AUC of 0.66 with a significant *P* value of 0.018, with best cut off 5.5 log₁₀ IU/

mL at which sensitivity, specificity, PPV, and NPV were 73%, 62%, 48%, and 45%, respectively.

Our results agree with a study done by Berg and others (2003) who reported that the resulting threshold of baseline HCV-RNA level after constructing an ROC curve for predicting virological response was 130,000 IU/mL and achieved an OR of 2.6 (95% confidence interval), a PPV, and an NPV of 71.7% and 50.7%, respectively.

According to our study, by using ROC curve analysis, baseline HCV-RNA level failed in differentiation between the rapid responders group (group I) and slow responder group (group II) (AUC of 0.61; $P=0.15$, NS).

To define predictive parameters for virological non-response, which allow for early discontinuation of therapy, we evaluated baseline factors such as type of IFN, ALT level, aspartate transaminase (AST) level, AFP level, activity grade, fibrosis score, and baseline HCV-RNA level of more than 600×10^3 IU/mL and positive HCV-RNA by PCR at week 4 in a univariate logistic regression model in which failure of response at end of treatment is the dependent factor and revealed that none of the previously mentioned parameters was a significant independent factor associated with failure of response to treatment.

Our results disagree with a study done by Oze and others (2013) who evaluated factors selected as significant for NR in the univariate analysis by multivariate logistic regression analysis such as age, grade of liver activity, stage of liver fibrosis, WBC count, platelet count, serum gamma glutamyl transferase level, IL28B genotype, and the magnitude of the decrease in HCV-RNA from baseline at week 4 in 2 models (model 1 included pretreatment factors and model 2 included pretreatment factors and virological response, which is the magnitude of decrease in HCV-RNA from baseline at treatment week 4). In model 1, IL28B genotype was the most powerful independent factor for NR (OR 39.75, $P=0.001$), apart from the degree of liver fibrosis (OR 10.31, $P=0.021$) and platelet count (OR 0.84, $P=0.01$). However, in model 2, the magnitude of decrease in HCV-RNA from baseline at week 4 was the most powerful independent factor for NR (OR 9.29, $P=0.001$), apart from the degree of liver fibrosis (OR 14.48, $P=0.004$). IL28B was not selected as a significant independent factor.

Also, our results disagree with a study done by Fried and others (2011) on 1,383 patients, encompassing genotypes 1–4, treated with PEG-IFN alpha-2a and ribavirin who reported that when an RVR was considered along with other baseline factors in a predictive model of SVR, significant predictors included were infection with HCV genotypes 2 or 3, younger age, lower baseline viral load, higher ALT ratio, lower creatinine clearance, and absence of advanced fibrosis on liver biopsy. Achievement of an RVR was the most important predictor of an SVR and was associated with the highest OR in this analysis.

Finally, the achievement of an ETR in HCV-infected patients in our study was mainly driven by the achievement of an RVR and EVR, with a great influence of low baseline serum HCV-RNA levels. While, on the level of univariate analysis none of the baseline parameters was a significant independent factor associated with failure of response to treatment.

The results of the current study demonstrated a high PPV of EVR for an ETR of 85%. The NPV of an EVR is more

robust for an ETR of 90%. Thus, an EVR is regarded as a robust indicator of treatment outcome and a 12-week stopping rule for patients is strongly evident.

Also, the results of the current study demonstrated a more robust PPV of RVR for an ETR of 88%. However, NPV of RVR is only 26.77% for ETR. Therefore, an RVR lacks reliability as a negative predictor and most likely will not result in a stopping rule; so, it does not eliminate the need for a 12-week assessment. However, it can be used to motivate patients to adhere for therapy.

Low baseline HCV-RNA level and high TSH level serve as important predictors of response to combined PEG-IFN and Ribavirin therapy in chronic HCV-infected patients. These results have important implications for predicting and managing response-guided combination antiviral therapies.

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Author Disclosure Statement

No competing financial interests exist.

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