Egyptian Journal of Medical Microbiology Volume 26 / No.1 / January 2017 67-75

ORIGINAL ARTICLE

Risk Factors for Chronic Hepatitis C Treatment Relapse with Sequence Based Genotyping among Egyptian Relapse Patients

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Key words:

APRI, SVR, Risk factors, Genotypes, Egypt

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ABSTRACT

Background: *Chronic Hepatitis C Virus (CHCV) infection in Egypt is a major health problem with a record of being the highest prevalence all over the world with a high incidence of new cases and relapses.* ***Objectives:*** *To monitor treatment response during and post therapy in Egyptian patients and to identify the factors that raises the risk of relapse including HCV genotypes distribution among relapsed patients.* ***Methodology:*** *A total of 125 CHCV-RNA positive patients on two interferon based regimens were followed for 48 week for viral response and relapse to therapy. Genotyping for relapsed patients was determined using direct sequencing and phylogenetic analysis of NS5B region of the HCV genome.* ***Results and Conclusions:*** *Among patients on first regimens, 16 (18.8%) had an overall viral relapse following end of treatment (EOT) and 11 (12.9%) of patients did not respond. In the second group, 8 (20.0%) got viral relapse and 6 (15.0%) were non responders. All relapsed strains fall in the genotype 4a category. In conclusion, old age, higher baseline viral RNA level, histological grade of fibrosis, AFP and APRI are factors that can increase the risk of developing relapse in patients undergo therapy.*

INTRODUCTION

Hepatitis C virus (HCV) is a major cause of liver disease worldwide with130–170 million people chronically infected and have a significant threat to develop cirrhosis and liver cancer [(1,2)]. The virus has a single-stranded RNA genome belonging to the Flaviviridae with approximately 9600 nucleotides. The RNA genome is structured in a coding region that holds one large open reading frame (ORF), flanked by non-translated regions (NTR) encoding a polyprotein precursor of about 3,000 amino acids. At least 10 different proteins are the products of the precursor cleavage; the structural proteins including core, E1, E2, and p7 and the non-structural including NS2, NS3, NS4A, NS4B, NS5A, and NS5B [(3)].

Genotyping is a useful tool for studying outbreaks and for better knowledge about the epidemiology and virological features of the virus in addition to its ability to predict the outcome of therapy[(4,5)]. Six major genotypes and several subtypes of the virus have been recognized [(6)] in addition to minor variants named as "quasispecies" are described[(7)]. The genotype 4 appears to be prevalent in the Middle East and Central Africa. Prevalence rates of HCV genotype 4 is estimated to be around 90% in Egypt where it has been reported to be associated with cirrhosis and a poor outcome to interferon (IFN) [(8,9)]. The HCV genotype 4 epidemic in this country was coupled with unhygienic parenteral intake of antischistosomal treatment campaigns, which were held till the mid-1980s [(10,11)].

Although many direct acting antiviral drugs (DAAs) found its way to clinical use, pegylated interferon α2a plus ribavirin (RBV) therapy were considered as a corner stone in the standard therapy used in a wide scale up with a rapid decline in viral load in the first 12 wk, for all HCV genotypes [(12,13)]. However; It is well recognized that their exclusive use is less effective in patients with genotypes 1 and 4 than in patients with genotypes 2 and 3 [(14)].

Pegylated IFN-α2a is produced by attachment of a 40 kDa branched polyethylene glycol moiety to IFN-α2a by a stable amide bond. It is characterized by prolonged absorption half-life, restricted volume distribution, and decreased clearance compared to standard interferon, which thus increase its therapeutic efficacy with less frequent doses [(15,16)]. Interferon works by binding to receptors on the cell surface that starts a complex cascade ending with rapid activation of gene transcription. This in turn may stop viral replication in infected cells [(17)]. Successful antiviral therapy with SVR which is undetectable HCV-RNA levels in the serum at end of treatment period (i.e., after 12, 24, 48 weeks) is the basis of stopping disease progression with its complications. However, a substantial number of patients do not achieve the proper response to the

peginterferon (pegIFN) plus ribavirin therapy. Several patterns of non-response are reported; a null response, a partial response, a breakthrough during treatment, and a breakthrough after achievement of end of treatment response (ETR) which is known as relapse "Relapse" is defined as a patient have experienced undetectable HCV RNA, and then become detectable again during or after the end of therapy [18].

Many factors appear to affect the success of pegylated interferon therapy like HCV genotype, base line viral load, presence of fibrosis or inflammation in the liver biopsy and the patient's body weight or body surface area [19]. In this study we try to evaluate several possible risk factors for viral relapse in Chronic HCV Egyptian patients.

METHODOLOGY

Study design and setting

This study was conducted between years from 2012 to 2014, on eligible chronic HCV patients recruited from Al-Ahrar Hospital, Egyptian ministry of health and population (MOHP) (located in Sharkia governorate, Egypt), a well-known center in Sharkia responsible for treatment of CHCV as a part of a national program for HCV treatment and eradication.

Target population and sampling

A total of 125 adult Egyptian patients chronically infected with HCV aged (18-60) years old who had serological, virologic and histopathological evidence of chronic HCV infection were enrolled in this study after obtaining a written and verbal consents from each patient.

Exclusion criteria

Decompensated liver cirrhosis, Hepatocellular carcinoma (HCC), prior anti-viral therapies, serious systemic disorders and current pregnancy or breastfeeding were considered as exclusion criteria.

Patient's classification

According to their weight, patients were divided into two groups;

- ***Group (A):*** patients weight ≥ 75 Kg; this group included 85 patients (67 males and 18 female) with age range (44.9 ± 9.5).
- ***Group (B):*** patients weight ≤ 75 Kg; this group included 40 patients, 31 males and 9 female with age range (39.9 ± 10.8). Cases were followed for 48 week and viral response and relapse was assessed.

Laboratory Procedures

Investigations performed as a part of the enrollment process included routine investigations; complete blood picture, liver enzymes; (AST, ALT), Creatinine, and Glucose, prothrombin time and activity. HCVAb, HBsAg, Thyroid-stimulating hormone (TSH) and Alpha-Fetoprotein (AFP) were tested by using Elecsys Cobas e-411 immunoassay analyzer (Roche molecular system, USA). Quantitative and qualitative determination of HCV-RNA were done by PCR using (Applied Biosystems StepOne RT-PCR, simple real-time PCR Systems, USA) and (COBAS Amplicor HCVv2.0, Roche molecular system. Branchburg, NJ, USA) respectively. Liver biopsies were obtained by ultrasonography guidance after checking the adequacy of the patient's coagulation profile. Metavir scoring method was used for histopathological assessment [20].

RT-PCR for sequencing and phylogenetic analysis for relapsed strains was done following the same protocol previously described by Fakhr et al. [21].

Management Protocol:

After a preliminary assessment, patients were treated using pegylated interferon at a dose of 180 µg per week (Peg-INFα-2a) subcutaneously plus ribavirin orally at a dose of 800-1200 mg daily as per body weight 800-1000 mg if ≤75 kg and 1000-1200 mg if ≥75 kg for 48 weeks. Clinical assessment, biochemical parameters, and viral RNA levels were done pretreatment and at weeks 4, 12, 24, and 48 of follow up.

Patients who were not fulfilling an early virologic response (EVR) which is ≥ 2 $\log_{10}$ reduction of serum HCV RNA after 12 weeks of therapy had discontinued treatment.

Statistical Methods

The Quantitative data was presented as mean ± SD; Qualitative data were illustrated as number and percent. Statistical analyses were analyzed by using SPSS software (Version 17.0). Multiple logistic regressions were used applied to assess the factors that raise the risk of CHCV relapse. P-value was considered significant if P-value was ≤ 0.05.

RESULTS

Data from a total of 125/150 treatment patients who achieved ETR were analyzed. Baseline characteristics of patients are shown in Table 1.

Table 1: Baseline characteristics of patients.

Demographic Features of the Studied Patients				
Variable	**Group (A) Peg-INF α-2a (180 µg/wk.) + RBV (1000-1200 mg/d) (n = 85)**		**Group (B) Peg-INF α-2a (180 µg/wk.) + RBV (800-1000 mg/d) (n= 40)**	
	No. (%)	**Mean ± SD**	**No. (%)**	**Mean ± SD**
Age(Years)		44.9 ± 9.5		39.9 ± 10.8
Gender :				
Male	67 (78.8%)	31 (77.5%)		
Female	18 (21.2%)	9 (22.5%)		
BMI(Kg/m2)		29.2 ± 3.4		24.3 ± 2.2
Laboratory Data of the Studied Groups				
Variable	**Group (A) (n = 85)**		**Group (B) (n= 40)**	
	Mean ± SD		**Mean ± SD**	
Glucose(mg/dl)	103.4 ± 28.9		97.8 ± 27.7	
Creat(mg/dl)	1.1 ± 0.40		0.90 ± 0.23	
AST(U/L)	42.7 ± 22.5		46.5 ± 20.3	
ALT(U/L)	48.4 ± 26.1		51.3 ± 22.3	
WBCs($x10^3/mm^3$)	6.9 ± 2.3		6.6 ± 1.9	
ANC($x10^3/mm^3$)	3.41 ± 1.13		3.33 ± 1.26	
Hb%(g/dL)	14.3 ± 1.6		13.9 ± 1.8	
PlT($x10^3/mm^3$)	202.5 ± 78.8		190.5 ± 58.3	
Prothrombin	81.7 ± 10.4		84.5 ± 10.8	
TSH(uIU/ml)	1.8 ± 1.10		1.6 ± 0.88	
HCVRNA(IU/ML)	703898 ± 853276		805876 ± 1144342	
AFP(ng/mL)	19.8 ± 22.6		19.1 ± 21.5	
APRI	2.3 ± 1.7		2.1 ± 1.6	
Histopathological Features of the Studied Groups				
Variable	**Group (A) (n = 85)**		**Group (B) (n= 40)**	
	No. (%)		**No. (%)**	
Fibrosis score :				
F0	17 (20%)		6 (15%)	
F1	32 (37.6%)		14 (35%)	
F2	12 (14.1%)		13 (32.5%)	
F3	16 (18.8%)		4 (10.0%)	
F4	8 (9.4%)		3 (7.5%)	

BMI: body mass index; **AST**: serum Aspirate Transaminase level; **ALT**: serum Alanine Transaminase level; **WBCs**: White blood count; **Hb%**: Hemoglobin; **PlT**: platelet count; **AFP**: Alpha-Fetoprotein; **APRI**: AST/Platelet Ratio Index; **HCV**: hepatitis C virus; **ANC**: Absolute neutrophil count; **TSH**: Thyroid-stimulating hormone; **Creat**: Creatinine, **Peg-INF α-2a**: Peginterferon α-2a; **RBV**: Ribavirin, **SVR**: sustained viral response, **F**: Fibrosis stages.

Virological Outcome:

Among 85 patients of group (A), 52 (61.2%) continued to have sustained virologic response (SVR), 16 (18.8%) had an overall viral relapse, 11 (12.9%) of patients did not respond at all and 6 (7.1%) stopped therapy before EOT either due to side effects of treatment or after being negative in the 4th week treatment dose. While in group (B) patients, 23 (57.5%) had SVR, 8 (20.0%) got viral relapse, 6 (15.0%) were non responders and 3 (7.5%) decided not to complete treatment Table 2.

The results showed that patients achieved EOT for 48 weeks had significantly higher chance to clear the virus and overall lower relapse rates where we can notice that the incidence of viral resolution after EOT for group A was 61.2% vs. 35.3, 52.9% for its rates after 12 and 24 weeks respectively (P = 0.02). In group B (SVR) incidence was 57.5% Vs. 25.0, 47.5% after 12, 24 week (P = 0.05) Table 2.

Table 2: Monitor of the patients response along and after EOT treatment.

Response Stages	group (A) (n = 85)			group (B) (n= 40)		
	12 week No. (%)	24 week No. (%)	48 week No. (%)	12 week No. (%)	24 week No. (%)	48 week No. (%)
EOT	83 (97.6%)	68 (80.0%)	57 (67.1%)	39 (97.5%)	31 (77.5%)	26 (65.0%)
VR	30 (35.3%)	45 (52.9%)	52 (61.2%)**	10 (25.0%)	19 (47.5%)	23 (57.5%)**
Relp	-	11 (12.9%)	5 (5.8%)	-	5 (12.5%)	3 (7.5%)
NR	11 (12.9%)			6 (15.0%)		
NCT	6 (7.1%)	-	-	3 (7.5 %)	-	-
P-value		0.02			0.05	

EOT: End of treatment**; VR**: Viral Resolution after specified time; **Relp**: Relapse; **NCT**: not complete therapy after 4 wk;****:**SVR; **NR**: non responders

Predictors of sustained viral response

The follow up of patient's response along treatment time revealed that almost 100 % of patients achieved rapid viral response (RVR) or extended rapid viral response (eRVR) and continued treatment were able to have SVR in both groups. While in group (A), only (57.7%) of patients with EVR and 58.3% of patients with partial early viral response (pEVR) continued to have SVR. In group (B) SVR was achieved in 64.3%of patients with EVR and 57.1% that have pEVR Table 3.

Table 3: Predictors of sustained viral response through the stages of treatment

Response Stages	Group (A) (n = 85)			Group (B) (n= 40)		
	No. (%)	SVR No. (%)	Relp No. (%)	No. (%)	SVR No. (%)	Relp No. (%)
RVR	32 (37.6%)	30 (93.8%)	Unknown*	11 (27.5%)	10 (90.9%)	Unknown*
eRVR	30 (35.3%)	30 (100 %)	0 (0.00) %	10 (25%)	10 (100%)	0 (0.00) %
EVR	26 (30.6%)	15 (57.7%)	11 (43.3%)	14 (35%)	9 (64.3%)	5 (35.7%)
pEVR	12 (14.1%)	7 (58.3%)	5 (41.7%)	7 (17.5%)	4 (57.1%)	3 (42.8%)
nEVR	11 (12.9%)	--	--	6 (15.0%)	--	--
SSE	4 (4.7%)	--	--	2 (5%)	--	--

RVR: rapid viral response; **eRVR**: extended rapid viral response; **EVR**: early viral response; **nEVR**: null early viral response; **pEVR**: partial early viral response; **SSE**: stop due to side effect; **EOTR**: end of treatment response; **SVR**: sustained viral response**; ***: Patients stopped therapy after this stage of treatment

Risk factors for relapse in patients:

The comparison between patients have SVR and those with relapse within both groups (A) and (B), revealed that individuals who experienced relapse were significantly different in pre-treatment stage of fibrosis, APRI, HCV-RNA level and AFP level than those who achieved SVR. Age was significantly different in group A only. Other factors including sex, body mass index, WBCs, Hb, platelet, serum ALT level, serum AST level, were similar between SVR and relapse patients in both groups Table 4.

Table 4: Univariate analysis showing baseline factors associated with sustained virological response and Relapse.

Factors	Group (A) Peg-INF α-2b (180 µg /wk) + RBV (1000-1200 mg/d) (n = 85)			Group (B) Peg-INF α-2b (180 µg /wk)+ RBV (800-1000 mg/d) (n= 40)		
	SVR (n=52)	Relapse (n= 16)	P-Value	SVR (n=23)	Relapse (n= 8)	P-Value
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
Age (Years)	**39.2 ± 11.8**	**46.0 ± 6.9**	**0.012***	**44.0 ± 10.7**	**43.2 ± 9.3**	**0.18**
BMI (Kg/m^2)	28.5 ± 3.3	30.2 ± 3.3	0.29	24.2 ± 2.4	24.3 ± 1.9	0.46
Glucose (mg/dl)	98.4 ± 23.9	103.7 ± 27.7	0.69	84.4 ± 18.9	93.1 ± 23.7	0.49
Creat(mg/dl)	1.0 ± 0.60	0.88 ± 0.45	0.44	0.92 ± 0.54	0.97 ± 0.47	0.38
AST (U/L)	30.1 ± 8.60	53.8 ± 18.5	0.19	40.2 ± 12.3	52.3 ± 16.9	0.28
ALT (U/L)	35.6 ± 11.8	49.3 ± 14,4	0.11	39.8 ± 17.7	55.8 ± 19.4	0.37
WBCs (x10^3/mm^3)	7.3 ± 2.5	6.3 ± 1.7	0.54	6.9 ± 1.9	6.1 ± 1.9	0.51
ANC (x10^3/mm^3)	3.01 ± 1.18	3.26 ± 1.22	0.51	2.87 ± 1.06	3.11 ± 1.16	0.46
Hb% (g/dL)	14.2 ± 1.8	14.5 ± 1.4	0.36	13.9 ± 1.6	13.4 ± 2.1	0.47
PIT (x10^3/mm^3)	212.9 ± 82.4	184.9 ± 70.3	0.31	191.3±49.8	189.4 ±71.8	0.54
Prothrombin	87.7 ± 9.4	82.5 ± 10.3	0.37	88.8 ± 11.4	87.5 ± 9.3	0.27
TSH (uIU/ml)	1.5 ± 1.00	1.3 ± 0.75	0.72	2.2 ± 0.90	2.9 ± 0.77	0.52
HCV RNA (IU/ML)	361341 ± 345173	951489 ± 1126030	0.001*	304326 ± 283623	1342100 ± 1504710	0.047*
AFP (ng/mL)	**5.9 ± 3.3**	**15.6 ± 6.6**	**0.019***	**5.6 ± 1.6**	**13.5 ± 5.7**	**0.021***
APRI	1.5 ± 0.6	3.5 ± 2.2	0.027*	1.4 ± 0.4	2.8 ± 2.1	0.016*
Factors	**SVR (n=52)**	**Relapse (n= 16)**	**P-value**	**SVR (n=23)**	**Relapse (n= 8)**	**P-value**
	No. (%)	**No. (%)**		**No. (%)**	**No. (%)**	
Sex :			0.86			0.39
Male	43 (82.7 %)	12 (75 %)		21 (91.3 %)	5 (62.5 %)	
Female	9 (17.3 %)	4 (25 %)		2 (17.3 %)	3 (37.5 %)	
Fibrosis score:			0.033*			0.01*
F0	14 (26.9 %)	0		5 (21.7 %)	0	
F1	29 (54.7 %)	0		11 (47.8 %)	1 (12.5%)	
F2	5 (9.4 %)	6 (37.5 %)		6 (26.0 %)	2 (25.0 %)	
F3	3 (5.7 %)	7 (43.7 %)		1 (4.5 %)	3 (37.5 %)	
F4	1 (1.9 %)	3 (18.8 %)		0 (0 %)	2 (25.0 %)	

BMI: body mass index; **AST**: serum Aspirate Transaminase level; **ALT**: serum Alanine Transaminase level; **WBCs**: White blood count; **Hb%**: Hemoglobin; **PIT**: platelet count; **AFP**: Alpha-Fetoprotein; **APRI**: AST/Platelet Ratio Index; **HCV**: hepatitis C virus; **ANC**: Absolute neutrophil count; **TSH**: Thyroid stimulating hormone; **Creat**: Creatinine, **Peg-INF α-2a**: Peginterferon α-2a; **RBV**: Ribavirin, **SVR**: sustained viral response, **F**: Fibrosis stages.

By binary logistic multivariate regression analysis using data in (Table 1), for group (A), older age ≥ 45 years, higher HCV- RNA level ≥ 780707 IU/Ml, higher AST/Platelet Ratio Index (APRI) ≥ 1.65, fibrosis score ≥ F2 and (Alpha-Fetoprotein) AFP ≥ 8.3 ng/ml were significantly correlated with occurrence of relapse, while in group (B), Higher HCV- RNA level ≥ 822031 IU/ml, higher APRI ≥ 1.57, fibrosis score ≥ F2 and AFP ≥ 8.8 were considered as independent risk factors for relapse**.**

HCV Genotypes Prevalent in Relapsed Patients

HCV genotypes were classified according to the nomenclature proposed previously by Simmonds et al.[22]. A total of 9 HCV PCR positive samples from relapse patients were collected and successfully genotyped by direct sequencing and phylogenetic analysis of the HCV NS5B gene. All relapsed strains fall in the genotype 4a which is the most prevalent genotype in the region**.**

Fig. 1: Phylogenetic tree of the NS5b region of samples. Neighbour-joining phylogenetic tree based on NS5B gene analysis from 31 Egyptian strains and 24 reference strains with different genotypes retrieved from GenBank.
The reference sequences were named after their accession numbers/ sub genotype. The study strains revealed to be responding to interferon therapy was marked by the sign○. The relapsed strains were marked by the sign●.

DISCUSSION

The Prevalence of CHCV infection with the high incidence of new cases and relapse rates after therapy across the Middle East and especially in Egypt requires a better understanding of the response to therapy and possible risk factors for relapse in these patients [(14)]. Along the time of the study, treatment with peginterferon alfa-2a or peginterferon alfa-2b, plus ribavirin, for 48 weeks was the approved regimen which was used on a wide scale in the national program for treatment and eradication of hepatitis held by the Egyptian Ministry of Health and Population [(23)].

Regarding the final treatment response, our study showed that the viral response rate was highest after 48 weeks (61.2%) versus 12 or 24 weeks treatment (35.3% and 52.9%) respectively in group A. Similarly in group B, highest rate (57.5%) of viral response was obtained after completion of whole course vs. 25.0% and 47.5% after 12 and 24 weeks treatment respectively.

The rates of ETR, SVR and overall relapse after 48 weeks treatment were 67.1%, 61.2%, 18.7%

respectively in group (A) and 65.0%, 57.5%, 20.0% respectively in group (B). Similar studies in different places revealed more or less comparable results. In Egypt, ***El-Raziky*** and his colleagues reported that ETR, SVR and relapse in patients treated with PEG-IFN-alfa-2a was 64.1%, 59.6%, and 27% respectively [23]. In line with our result, another study in Saudi Arabia, reported that ETR, SVR, relapse in patients treated with PEG-IFN-alfa-2a was 66.7%, 61.5%, and 7% respectively[24].

On the contrary, many other studies originating from our region showed significantly different results for the rates of SVR. Significantly lower results between 40.9% to 56.0%[23,25, 26,27] or significantly higher between the 68.0% to 73.0% both they used PegIFN-α2a or PegIFN-α2b for treatment patients with HCV [28, 29].

Studies conducted in Europe have shown that the SVR rates in Europeans or Africans with chronic HCV-4 treated with PEG-IFN-alpha (2a or 2b) plus ribavirin are lower (40.9%-63.0% or 46.7%-60.7%) than those achieved in studies conducted in the Middle East[30,31]. The analysis of studies conducted between Europeans and Egyptian patients with chronic HCV-4 showed an overall better response in Egyptians infected with the 4a subtype than those European patients infected [32,33]. Several factors could be considered as reasons for different results for SVR. More or less strict exclusion criteria, selection of specific group as diabetic patients, different study design or different locations with variable subgenotypes or patients' genetic elements could have impact on the result.

Early prediction of virological response for therapy can help the clinicians to identify patients who are unlikely to have a sustained response and take the decision to discontinue treatment, saving patients from the side effects and cost of additional therapy [25]. Regarding the predictors of SVR through the stages of treatment in this study, a rapid viral response (RVR) by (week 4 or week 8) was the best predictor for SVR. 93.8% and 90.9% of group A and group B of patients who had (RVR), achieved sustained virological response. Supporting our finding in Egypt, Khattab and his colleagues reported that among 131 patients, the overall SVR rates was 60.3 % and 100 % of those had RVR achieved by EOT sustained virological response[33].

The important characteristics observed among those who achieved a RVR were younger age, low pretreatment viral load, lower BMI, lower baseline histologic grade and lower APRI ratio. This observation agrees with another Egyptian study which revealed that younger age, lower baseline histologic grade and female gender were more likely to attain RVR and EVR than males [27]. Consistent with our results also regarding age and pretreatment inflammatory grade, in a study from Saudi Arabia, six variables were introduced which were significantly related to SVR (younger age, non-diabetics, higher serum albumin, less pretreatment inflammatory grade, infected with genotypes 2 or 3, and treatment-naïve patients). However; in this study blood sugar, serum albumin were not found to be significantly related to SVR, no variability in genotypes was observed and all patients were naïve [26]. Using univariate logistic regression analysis, serum alpha fetoprotein (AFP) was found to be an independent variable associated with failure of achieving SVR. It is supported by finding of El-Raziky et al and Gad et al in which patients with lower base line AFP were most likely to achieve SVR [23, 25].

Regarding HCV genotype, *Medrano et al* reported that HCV relapse is more common in patients infected with HCV genotypes 1–4 than with genotypes 2–3 [34]. However, both genotypes 2-3 are very rarely reported in Egypt. In this study all relapsed cases were found to be genotype 4a which is the already prevalent genotype in Egypt [21]. The ethnicity also has been reported as a risk factor in relapse. Zeuzem reported that African-American ethnicity in addition to genotype 1, high HCV RNA concentrations, advanced duration of infection, histologically advanced liver disease, increased bodyweight and previous treatment relapse were among the factors that are associated with a less favorable outcome [35].

In Korea, Su Rin Shin and his colleagues reported that risk factors for relapse were low adherence to Peg-INF in genotypes 2 and 3 which was not observed in present study. However; similar to our results, older age (≥ 50 years) and higher baseline HCV-RNA level (≥ 2000000 IU/mL) were the main risk factors for relapse in genotype 1 [36].

Some other risk factors like HIV Co-infection [37], BMI > 27 Kg/m^2, platelets counts<140.000 mm^3[38], IL28B non CC [39], and a suboptimal dose of ribavirin were already reported [40]. However over weight was not found as a significant risk factor in HCV genotypes 2/3[41].

In this study, high AST/Platelet Ratio Index (APRI) was found to be significantly associated with relapse. APRI was reported as a serological marker that has satisfactory sensitivity, specificity with a high predictive value to check the progress of chronic hepatitis C. Also, it can help in determination of the correct time for treatment initiation in the absence of a biopsy with accuracy comparable to that of a biopsy. It has been accepted as a screening test for evaluating histologic liver fibrosis [42,43]. Low APRI score and no sign of chronic liver disease in chronic hepatitis C patient indicate less probability histologic liver fibrosis with no need to perform liver biopsy [44].

In conclusion, old age with higher baseline viral RNA level, histological grade of fibrosis, AFP and APRI are factors that can increase the risk of developing relapse in patients undergo therapy.

Financial disclosure:

No financial interests related to the material in the manuscript.

Acknowledgments

Funding of investigations and treatment expenses were provided by the Egyptian Ministry of Health and Population (MOHP) as a part of a national program of HCV treatment and eradication. We thank all the doctors for their important contributions and their continuous support, instructions, advice and tips during the supervision of this work.

REFERENCES

1. Lucinda K, Porter KN. et al. A Guide to hepatitis C Treatment side effect management. San Francisco CA. 2010; 94142-97037.
2. Lavanchy D. The global burden of hepatitis C. Liver Int. 2009; 29:74–81.
3. Moradpour D, Penin F, Rice CM. et al. Replication of hepatitis C virus. Nat Rev Microbiol. 2007; 5:453-63.
4. Simmonds P. Genetic diversity and evolution of hepatitis C virus–15 years on. J Gen Virol. 2004; 85: 3173-3188.
5. Lyra AC, Ramrakhiani S, Bacon BR, Di Bisceglie AM. et al. Infection with hepatitis C virus genotype 4 in the United States. J ClinGastroenterol. 2004; 38: 68-71
6. Simmonds P, Bukh J, Combet C, Deleage G, Enomoto N, Feinstone S. et al. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 2005; 42: 962-73.
7. Farci P, Purcell RH. Clinical significance of hepatitis C virus genotypes and quasispecies. Semin Liver Dis. 2000; 20: 103-126
8. El-Zayadi A, Simmonds P, Dabbous H, Prescott L, Selim O, Ahdy A. Response to interferon-alpha of Egyptian patients infected with hepatitis C virus genotype 4. J Viral Hepat. 1996; 3:261–264.
9. Shobokshi O, Serebour FR, Skakni L, Al-Jaser N, Tantawe AO, Sabah A, Dinish T, Qatani T, Sandokji A, Al-Blowi A. et al. Peg-IFN Alfa-2a (40kda) As A Monotherapy or In Combination With Ribavirin Significantly Improve End Of Treatment Response Rate. In: HCV Genotype 4 Chronic Active Hepatitis (CAH) Patients. Boston MA Hepatology. 2002; 36:2.
10. Frank C, Mohamed MK, Strickland GT,Lavanchy D, Arthur RR,Magder L.S. et al. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet. 2000; 355:887-91.
11. Tanaka Y, Agha S,Saudy N,KurbanovF,Orito E, Kato T. et al. Ex-ponential spread of hepatitis C virus genotype 4a in Egypt. J MolEvol. 2004; 58:191–5.
12. Shiffman ML.. Pegylated interferons: what role will they play in the treatment of chronic hepatitis C. Gastroenterol Rep. 2001; 3: 30-37
13. Hadziyannis SJ, Papatheodoridis Gv. Recent Peginteferonand Ribavirin Combination Trials. Current Hepatitis Reports. 2004; 3: 30-37
14. Sherman M. Response to Pegasys and Regular Interferon A-2a for Individuals with Chronic HCV and Genotype 4. Virologic Response to Pegasys with Genotype 4 In Small Study Group. Hepatology. 2002; 1: 1A-32A
15. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med. 2002; 347: 975-982
16. El-Makhzangy H,Rekacewicz C,Shouman SI, Mohamed HN,Esmat G, Ismail A. et al. Combined pegylated interferon alpha-2a and ribavirin in treatment of chronic hepatitis C in Egypt ANRS (1211 Trial). In: 4th Annual Meeting of the European Association for the Study of the Liver. 2005;13:17.
17. Kamal SM, Moustafa KN, Chen J, Fehr J, Abdel Moneim A, Khalifa KE. et al. Duration of peginterferon therapy in acute hepatitis C: a randomized trial. HEPATOLOGY 2006;43:923-931.
18. Shiffman ML. Optimizing the current therapy for chronic hepatitis C virus: peginterferon and ribavirin dosing and the utility of growth factors. Clin Liver Dis. 2008; 12: 487–505.
19. Dibisceglie A, Rustgi V, Thuluvath P, davis M, Ghalib R, Lyons M, Ondovik M, Lopez-Talavera J, Hamzeh F. et al. Pharmacokinetics and pharmakodynamics of pegylated interferon-alpa2a with ribavirin in treatment naive patients with genotype 1 chronic hepatitis. 55th AASLD 2004. Boston MA. Abstract LB-18
20. Bedossa P, Poynard T. The METAVIR cooperative study group. An algorithm for the grading of activity in chronic hepatitis C. Hepatology. 1996; 24: 289–293.
21. Elsadek A F.; Mahmoud Reza Pourkarim, Piet Maes, Amal Hassan Atta, Ayman Marei, Magda Azab, and Marc Van Ranst, et al. Hepatitis C Virus NS5B Sequence-Based Genotyping Analysis of Patients From the Sharkia Governorate Egypt. Hepat Mon. 2013; 13: e12706.
22. Simmonds P,Bukh J,Combet C,Deleage G,Enomoto N,Feinstone S,halfon P,Inchauspe G,Kuoken C. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatol. 2005; 42:962-973.
23. El-Raziky M, Fathalah WF, El-Akel WA.et al. The effect of peginterferon alpha-2a vs. peginterferon alpha-2b in treatment of naive chronic HCV genotype-4 patients: a single center Egyptian study. Hepat Mon. 2013; 13: e10069.
24. Dahalan Y, Ather HM, Al-ahmadi M, Batwa F, Al-hamoudi W. et al. Sustained virological response in a predominantly hepatitis C virus genotype 4 infected population. World J Gastroenterol. 2009; 15: 4429-4433.
25. Gad RR, Males S, El-Makhzangy H. et al. Predictors of a sustained virological response in patients with genotype 4 chronic hepatitis C. Liver Int. 2008; 28: 1112–9.
26. Al-Ashgar H, Khan MQ, Helmy A, Al Swat K, Al Shehri A, Al Quaiz M. et al. Sustained virologic

response to peg-INF alpha 2a and Ribavirin in 335 patients with chronic hepatitis C: a tertiary care center experience. Saudi J Gastroenterol. 2008; 14:58-65.

27. Kamal SM, El Kamary SS, Shardell MD, et al. Pegylated interferon alpha-2b plus ribavirin in patients with genotype 4 chronic hepatitis C: the role of rapid and early virologic response. Hepatology. 2007; 46: 1732–40.
28. Kamal SM, El Tawil AA, Nakano T, He Q, Rasenack J, Hakam SA. et al. Peginterferon alpha-2b and ribavirin therapy in chronic hepatitis C genotype 4 : impact of treatment duration and viral kinetics on sustained virological response. Gut. 2005; 54:858-866.
29. Hasan F, Asker H, Al-Khaldi J, Siddique I, Al-Ajmi M, Owaid S. et al. Peginterferon alfa-2b plus ribavirin for the treatment of chronic hepatitis C genotype 4. Am J Gastroenterol. 2004; 99: 1733-1737.
30. Sanchez-Tapias JM, Diago M, Escartin P, Enriquez J, Romero-Gomez M, Barcena R. et al. Peginterferon-alfa2a plus ribavirin for 48 versus 72 weeks in patients with detectable hepatitis C virus RNA at week 4 of treatment. Gastroenterology. 2006; 131:451-460.
31. Elefsiniotis IS, Vezali E, Mihas C, Saroglou G. et al. Predictive value of complete and partial early virological response on sustained virological response rates of genotype-4 chronic hepatitis C patients treated with PEGinterferon plus ribavirin. Intervirology. 2009; 52:247–251.
32. Roulot D, Bourcier V, Grando V. et al. Epidemiological characteristics and response to peginterferon plus ribavirin treatment of hepatitis C virus genotype 4 infection. J Viral Hepat. 2007; 14: 460-7.
33. Khattab M, Eslam M, Sharwae MA, Shatat M, Ali A, Hamdy L. et al. Insulin resistance predicts rapid virologic response to Peginterferon/Ribavirin combination therapy in hepatitis C genotype 4 patients. Am J Gastroenterol. 2010; 105:1970-1977.
34. Medrano J, Pablo B, Salvador R, Paula T, Violeta R, Eugenia V, Pablo L, Antonio M, Javier G, Inmaculada J, Luz M, Vincent S. Rate and Timing of Hepatitis C Virus Relapse after a Successful Course of Pegylated Interferon plus Ribavirin in HIV-Infected and HIV-Uninfected Patients. Clin Infect Dis. 2009; 49:1397-1401.
35. Zeuzem S. Heterogeneous virologic response rates to interferon-based therapy in patients with chronic hepatitis C: who responds less well? Ann Intern Med. 2004;140: 370-81.
36. Shin SR, Sinn DH, Gwak GY, Choi MS, Lee JH, Koh KC, Yoo BC, Paik SW. Risk factors for relapse in chronic hepatitis C patients who have achieved end of treatment response.J Gastroenterol Hepatol. 2010; 25:957-963.
37. Hughes CA, Shafran SD. Treatment of hepatitis C in HIV coinfected patients. Ann Pharmacother. 2006;40:479-489.
38. Basso M, Giannini EG, Torre F, Blanchi S, Savarino V, Picciotto A. Elevations in alanine aminotransferase levels late in the course of antiviral therapy in hepatitis C virus RNA-negative patients are associated with virological relapse. Hepatology 2009; 49: 1442-8.
39. Mangia AN, Minerva D,Bacca R,CozzolongoE, Agostinacchio F,Sogari F,Vinelli G, Scotto GL,. Ricci M, Romano V,Carretta D,Petruzzellis V,Piazzolla F,Spirito A. 8 Factors Predictive Of Relapse In Genotype 2 And 3 Pts Treated For 12 Weeks With Pegifn Alfa 2b And Weight Based Ribavirin Combination. Journal of Hepatology , Volume 48 , S6 Conference Reports for NATAP, 2008. http://www.natap.org/2008/EASL/EASL_08.htm
40. Foster GR,Strasser S, Christensen C, Brainard DM, Symonds WT, McHutchison JG, Conway B, Crespo L, Zeuzem S. et al. Sofosbuvir-based regimens are associated with high SVR rates across genotypes and among patients with multiple negative predictive factors. 49th Annual Meeting of the European Association for the Study of the Liver, abstract O66, London, 2014.http://www.aidsmap.com/Sofosbuvir-works-well-despite-multiple-negative-predictive-factors/page/2848419/
41. Jacobson IM, Brown RS, Freilich B, Afdhal N, Kwo PY, Santoro J, Becker S, Wakil AE, Pound D, Godofsky E, Strauss R, Bernstein D, Flamm S, Pauly MP, Mukhopadhyay P, Griffel LH, Brass CA, WIN-R Study Group. Peginterferon alfa-2b and weight-based or flat-dose ribavirin in chronic hepatitis C patients: a randomized trial. Hepatology. 2007;46:971–81.
42. Borsoi V, Kioko T, Diva C, Collarile Y, Betty G, Edna S. Use of AST platelet ratio index (APRI Score) as an alternative to liver biopsy for treatment indication in chronic hepatitis C. An Hepatol. 2009; 8:26-31.
43. Leroy V, Hilleret MN, Sturm N, Trocme C, Renversez JC, Faure P, Morel F, Zarski JP. Prospective comparison of six non-invasive scores for the diagnosis of liver fibrosis in chronic hepatitis C. J Hepatol. 2007; 46:775-82.
44. Prechachaisurat T, Sakdapanpaisan S, Thirayost N, untipanichteerakul S, Piyanirun W. Use of AST Platelet Ratio Index Score (APRI Score) for Predicting Histologic Liver Fibrosis in Chronic Hepatitis C Patients at Phramongkutklao Hospital and BhumibolAdulyadej Hospital. Gastroenterol 2012, 13: 15-18.