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Monitoring the Treatment of Hepatitis C Viral Infection by Molecular Techniques

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Abstract

The prevalence of Hepatitis C viral infection in Egypt is the highest rate in the world. It is a major public health problem in Egypt. The objective of this study was to detect the clearance of HCV-RNA in Egyptian patients who were recommended for combination therapy of pyglated interferon α 2a and ribavirin (PEG-interferon/Ribavirin). Ninety-five positive HCV-IgG cases were tested for HCV-RNA at baseline and at weeks 4, 12, 24, 48 and 72 of treatment with PEG-interferon α 2a/Ribavirin. The correlations between the viral parameters during and after treatment were evaluated. Patients recommended receiving PEG-interferon 2a/ribavirin combination therapy showed high response rate determined as 71.9% achieved Sustained Virological Response (SVR) from total patients, and 71.4% from patients who received 48 weeks therapy. In general, the employment of TMA provided us the accuracy of results with confidence in our work. There was a significant relation ($p < 0.001$) between results of Transcription mediated amplification (TMA) and response to therapy that indicate to positive correlation. The development of sensitive accurate assays for HCV-RNA detection and quantification is necessary to improve not only the assessment of the response to antiviral therapy but also our understanding of the mechanism underlying antiviral resistance.

Keywords

Monitoring, Hepatitis C Infection, RNA, Treatment, Molecular Techniques

1. Introduction

The best and standard approach to monitoring for treatment affectivity consists of continual measuring of quantitative HV-RNA levels. Hepatitis C viral infec-

tion is a serious medical problem in Egypt and it has a devastating impact on the Egyptian economy. It is estimated that over 18% of Egyptians are infected by the virus [1] [2]. So Egypt considered as one of the world's highest prevalence of HCV with a majority of genotype-4 infection [3] [4]. Nucleic acid tests (NATs) directly detect the presence of HCV RNA using a combination of amplification and detection techniques. NATs are classifies into qualitative and quantitative tests to detect HCV RNA. In deed the response to the therapy and its successfulness are very important to be detected using more sensitive and specific qualitative and quantitative tests. Transcription mediated amplification (TMA) is the most sensitive qualitative HCV RNA assay, of detection limit that start from 5 IU/ml and sensitivity more than 98% to confirm viremia and to detect clearance of viremia during and after the completion of the treatment [5]. The branched DNA (bDNA) is the first Food and Drug administration (FDA) approved technique to quantify HCV RNA. The method differ from RT-PCR tests, in that the detection signals is amplified rather than target RNA. The third generation assay (bDNA 3.0) has a high dynamic range of lower detection limit of 615 IU/ml and an upper range of 7.7 million IU/ml. It is highly reproducible and the specificity range from 96% to 98% [6]. So the previously mentioned nucleic acid tests (NATs) are very important combined with each other's to monitor clearance of HCV-RNA in therapeutic treated patients with a global TMA technology Confirmation. This study aimed to investigate the clearance of HCV-RNA in Egyptian patients who were recommended for combination therapy of pyglated interferon α 2a and ribavirin (PEG-interferon/Ribavirin). This enables us to evaluate the different responses to the combination therapy of PEG-interferon/Ribavirin as a good used regimen in treatment of chronic HCV in Egyptian patients.

2. Subjects, Materials and Methods

2.1. Study Population

This study (approved by the Ethical Committee of Ain Shams University) included 85 HCV-Ab positive cases collected from Cairo, Egypt (El Hussein and Sayed Galal hospitals), Al Menia (El Menia hospital) and Al Daqahlya (Al Mansoura general hospital) from October 2017 to March 2019. Ethics approval was obtained for the study and informed consent forms were signed by patients. The 85 cases included 45 females and 40 males between 18 - 65 years of age. The subjects were divided into two groups: First group were positive HCV-Ab and titre of HCV-RNA is less than 615 IU/ml (n = 28) and Second group were positive HCV-Ab and titre of HCV-RNA is more than 615 IU/ml (n = 57). Blood samples were drawn from all study participants and serum samples were separated and stored at −80˚C until further testing.

2.2. Detection of HCV IgG Antibodies (Abs)

Antibodies were estimated in serum samples by the enzyme-linked immunosor-

bent assay (ELISA) technique using commercially available HCV-IgG kit ETI-AB-HCV K-4 (N0146, N0147), DiaSorin, Spain.

2.3. HCV-RNA Qualitative Assay by Transcription Mediated Amplification Technology (TMA)

HCV-RNA were estimated by Transcription mediated amplification Technology (TMA) using VERSANT® HCV RNA Qualitative assay, Gen-Probe incorporated for: Siemens Medical Solutions Diagnostics (Trrytown, USA).

2.4. HCV-RNA Quantitation by Branched DNA (bDNA) Technology

This technique was applied for all samples using VERSANT® HCV RNA 3.0 Assay (b DNA), Siemens Medical Solutions Diagnostics (Trrytown, USA).

2.5. Statistical Analysis

All statistical analyses were performed using the SPSS V.12 statistical software program. The statistical significance of difference was considered when $p \leq 0.05$.

3. Results

3.1. Study Population Characteristics

Eighty-five Cases were considered newly identified HCV-Ab Positive.14.1% of whom were between 18 - 40 years old, 47.1% were between 41 - 56 years old, and 35.1% 56 years or more **Table 1**. According to the results obtained by b-DNA assay based on the baseline value of 615 IU/ml of HCV-RNA, the study population was divided into 2 groups. The first group, representing (33%) of the total, included 28 with positive HCV-Ab and HCV-RNA < 615 IU/ml (11 females and 17 males), whose age range was between 18 - 50 years, with a mean age of 36.1 ± 8.2 years. Those patients were defined as viral self-clearers. Accordingly, those patients of first group were also excluded from our study. Since they showed undetectable titre of HCV-RNA < 615 IU/ml by using b-DNA and also they gave negative results with TMA technique (the basis of TMA detection limit 5 IU/ml) (**Figure 1**). The Second group, representing (67%) of the total, included 57 with positive HCV-Ab and HCV-RNA > 615 IU/ml (34 females and 23 males), whose age range was between 25 - 65 years, with a mean age of 42.03 ± 11.1 years. All cases of group two were recommended for treatment PEG-interferon α 2a therapy (180 ug) for the most majority of the patients and (160 ug) for the minority of the patients, once a week dose combined with ribavirin (1000 to 1200 mg) for all patients daily by their physicians.

All patients of group two were subjected to quantitative assay (b-DNA) and qualitative assay (TMA) according to the following protocol:

- Pre-therapy (assays performed before treatment
- Time intervals (assays performed at several intervals of treatment 4, 12, 24 and 48 weeks).
- Post-therapy (assays performed at 72 week of treatment).

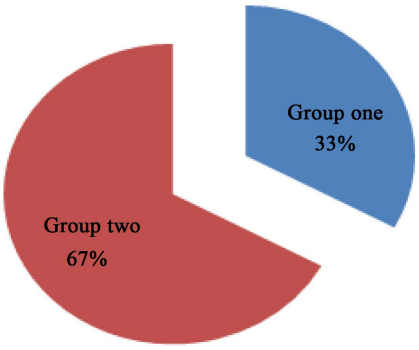

Figure 1. Percent of group one cases with contrast to patients of group two.

Table 1. Distribution of participants' sociodemographic and Clinical characteristics.

Sociodemographic characteristics	n	%
Age		
18 - 40	12	14.1
41 - 56	40	47.1
>56	33	38.8
sex		
male	40	47
female	45	53
Place of birth		
Urban area	52	61.1
Rural area	33	38.9
Clinical characteristics		
ABO group		
A	15	17.6
B	10	11.8
AB	12	14.1
O	48	56.5
Positive HCV-Ab (n = 85)		
HCV-RNA < 615 IU/ml	28	33
HCV-RNA > 615 IU/ml	57	67

3.2. Response of Patients to Therapeutic Treatment

The lowest percentage of response 42.1% (24/57) was reported at week 4 from therapy in rapid virological response (RVR) patients. The highest percentage of response 93% (53/57) was achieved at week 12 in early virological response (EVR) patients. The high percentage of response 82.2% (46/56) at week 48 was obtained in end of treatment response (ETR) patients. The global high percentage of sustained virological response (SVR)71.4% (40/56) patients was reported for patients who recommended to complete 48 weeks treatment, SVR was

achieved by 40 patients after exclusion of the single patient who stopped therapy at week 24 as a responder (shorter duration of therapy) Figure 2. SVR referred actually to the final response to the therapy.

3.3. Correlation between HCV-RNA Quantitative by b-DNA and Final Sustained Response to Therapy (SVR)

The relation between patients' response to therapy and HCV-RNA quantification value at week 4, 12 was statistically significant (P-value < 0.05).

At week 4, 41 out of 57 (72%) were sustained responder to therapy and 16 out of 57 (28%) were sustained non-responder. HCV-RNA quantification value < 615 IU/ml at week 4 was detected in 24/57 patients, while 33 out of 57 were HCV-RNA quantification value > 615 IU/ml. the high sustained response to therapy was reported for patients of HCV-RNA < 615 IU/ml than those of HCV-RNA > 615 IU/ml. At week 12, 41 out of 57 (72%) were sustained responder to therapy and 16 out of 57 (28%) were sustained non-responder. HCV-RNA quantification value < 615 IU/ml at week 12 was detected in 45/57 patients, while 12 out of 57 were HCV-RNA quantification value > 615 IU/ml. the high sustained response to therapy was reported for patients of HCV-RNA < 615 IU/ml than those of HCV-RNA > 615 IU/ml (Table 2).

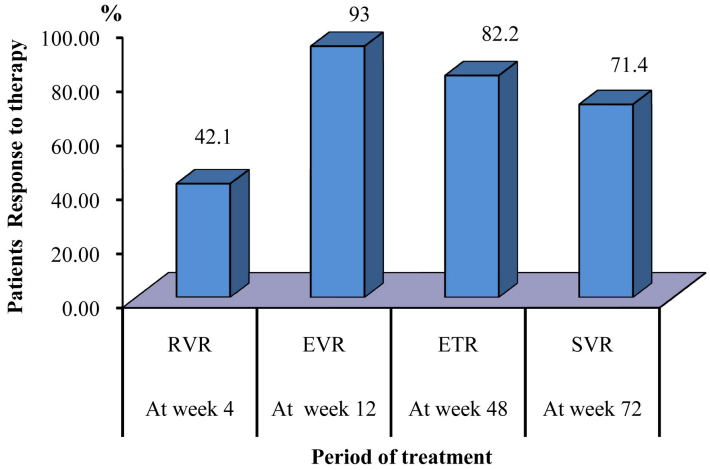

Figure 2. Diagrammatic representation of different patients' response behaviors to PEG-interferon α2a/RBV therapy.

Table 2. The correlation between quantitative values of HCV-RNA applied by b-DNA at week 4 and 12 and patients' sustained response or non-response to therapy.

	HCV-RNA (b-DNA)	sustained responder to therapy		sustained non-responder	
		No.	total	No.	total
At week 4	<615 IU/ml	21/24 (87.5%)	41/57 (72%)	3/24 (12.5%)	16/57 (28.1%)
	>615 IU/ml	20/33 (60.6%)		13/33 (39.4%)	
At week 12	<615 IU/ml	35/45 (77.7%)	45/57 (78.9%)	10/45 (22.2%)	16/57 (28.1%)
	>615 IU/ml	6/12 (50%)		6/12 (50%)	

3.4. Correlation between Qualitative Determination of HCV-RNA by TMA and Sustained Final Response to Therapy (SVR)

The relation between patients' response to therapy and qualitative detection of HCV-RNA by TMA method at weeks 24, 48 and 72 was statistically significant (P-value < 0.001). At week 24 of treatment, 49 out of 54 (90.7%) were negative HCV-RNA. In these cases, 41 patients (75.9%) were highly sustained response to therapy. the sustained response to therapy could not be monitored in patients with positive HCV-RNA, so the remaining 5 positive patients were sustained non-responder. At week 48 of treatment, 47 out of 54 (87.03%) were negative HCV-RNA. In these cases, 41 patients (75.9%) were highly sustained response to therapy. the sustained response to therapy could not be monitored in patients with positive HCV-RNA, so the remaining 8 positive patients were sustained non-responder. At week 72 of treatment, the highest sustained response rate was associated with the negativity of HCV-RNA results. The total patients enrolled for HCV RNA detection at week 72 were 46 patients. 41 out of 46 (89.1%) were negative HCV-RNA. In such patients the high sustained response to therapy was reported in 86.96% (Table 3). In positive HCV-RNA patients, the sustained response to therapy could not be reported.

4. Discussion

Our study proceeded by selection of 85 positive HCV-Ab patients. HCV-RNA quantitative by b-DNA was performed firstly for all positive HCV-Ab patients, followed by TMA qualitative assay. The later technique has applied to confirm viremia more accurately [5] [7]. The results were obtained as <615 IU/ml below the detection limit in 28/85 patients (***First group***) and >615 IU/ml in the rest majority (57/85) patients (***Second group***). In the first group, the results were negative for HCV-RNA by TMA. The results on the basis of TMA detection limit 5 IU/ml makes it the most promising tool indicating the clearance of viremia with confidence [3] [8]. The patients of group one, they defined as viral self-clearers, representing 32.95%. This fall in agreement with studies, Seeff (2002) [9], Wong and Lee (2006) [10] and Ponziani *et al.* [11] reported that 15% - 50%

Table 3. Correlation between qualitative detection of HCV-RNA by TMA at week 24, 48 and 72 and patients' sustained response or non-response to therapy.

	HCV-RNA (TMA)	sustained responder to therapy		sustained non-responder	
		No.	total	No.	total
At week 24	negative	41/49 (83.7%)	41/54 (75.9%)	8/49 (16.3%)	13/54 (24.07%)
	positive	0/5		5/5 (100%)	
At week 48	negative	41/47 (87.2%)	41/54 (75.9%)	6/47 (12.8%)	13/54 (24.07%)
	positive	0/7		7/7 (100%)	
At week 72	negative	40/40 (100%)	40/49 (86.9%)	0/40	6/46 (13.04%)
	positive	0/6		6/6 (100%)	

clear the infection. The mechanism of clearance of viremia in those patients may be attributed to their immune response against infection and may be explained due a strong multi-specific CD4+ T cell response produced early in infection that is associated with viral clearance and disease resolution, Whereas, a narrowly focused and delayed response is associated with chronic infection [12] [13]. Also, a strong multi specific CD8+ T cell response produced early in infection is associated with viral clearance [14].

In this study, the majority of the patients (57/85) were recommended to receive combination therapy of peglated interferon plus ribavirin (PEG-interferon α 2a/RBV). The current impression that the patients infected with HCV genotype 4 are difficult to treat and that they respond poorly to interferon therapy was made based on earlier studies where conventional interferon-alpha (INF-α) was used alone [15]. On the other hand, studies that used PEG-interferon/RBV on genotype 4 patients showed better results [16]. Those authors recommended treatment for patients with HCV genotype 1 and 4 with PEG-interferon plus RBV for 48 weeks**.** The data of this study were monitored at baseline and at weeks 4, 12, 24, 48, and 72 of follow up According to the study that carried out by Al Ashgar *et al*. [16]. In studying of virological response; the viral load was determined quantitatively by b-DNA as the main technique to determine rapid virological reponse (RVR) and early virological response (EVR) at weeks 4 and 12 from therapy respectively. The primary response to the therapy represented by significant decline in viral load [17]. At week 24 all patients who previously achieved EVR (Responders), those patients were recommended to assess the presence of HCV-RNA with a sensitive technique in which the lower detection limit: 50 IU/ml or less at week 24 from therapy [17]. So, TMA qualitative assay was applied as the best detector for HCV-RNA, also it was used as qualitative technique at end of treatment at 48 week, and at week 72 to determine patients achieved end of treatment response (ETR) and sustained virological response (SVR), respectively [3] [18] [19]. In weeks 24, 48 and 72, b-DNA was applied to non-responders for its minor role with non-significant value in treatment intervals of treatment. It just applied to detect the variation in HCV-RNA titers.

In the results of this study, the rapid virological response (RVR) was found in 24/57 patients (42.1%). These results differ from the study by Martin-Carbonero *et al*. [20] who reported 26.1% RVR in all HCV patients. The patients achieved RVR (24 patients) could achieve SVR (21 patients) by 87.5%. The data of this study similar to that reported by Martin-Carbonero *et al*. [20] who stated that patients achieved RVR could achieve SVR by 83% for HCV cases.

Early viral response (EVR) in this study was shown in 53/57 patients 93% similarly, Al Ashgar *et al*. [16] found EVR in 75.3% in their patients. In other studies carried out on the effect of INF plus RBV, other study found that EVR was achieved by 50% and 43% respectively [21]. These revealed the superiority of PEG-interferon/RBV than the conventional interferon during therapy. In this study, the patients achieved EVR (53 patients) could achieve SVR (41 patients) by 76%. Treatment must be continued when there is a 2-log drop in HCV-RNA

level, or when HCV RNA is undetectable at week 12, in these patients HCV-RNA detected at week 24 by the most sensitive qualitative technique [17].

This study reveals that there were 3 patients recommended to stop therapy at week 24; 2 patients as non-responders and one patient as responder without compromising SVR characterized by low baseline viral load 36.780 IU/ml, undetectable viremia at weeks 4 and 12, and negative HCV RNA by TMA at week 24, indicating the actual clearance of viremia [20].

Sustained virological response (SVR) was achieved in this study by 40/57 patients (71.42%) that vary from results reported by Martin-Carbonero *et al*. [20] who showed 32.6% SVR, and Wong and Lee [10] summarized results of randomized controlled trials by several authors Lindsay *et al*. [22] and Derbala *et al*. [15] which gave also a range of 33% - 77% SVR. Also, this result was in agreement with previous studied by Zekri *et al*. [23] as their patients with genotype 4 showed SVR rates of 68% when treated with PEG-interferon/Ribavirin combination treatment for 48 weeks.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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