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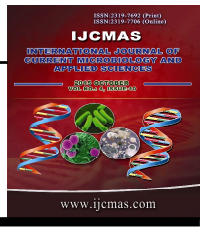

Original Research Article

Is Vitamin D Predictor of Response to Pegylated Interferon and Ribavirin treatment in Chronic Hepatitis C Egyptian Patients?

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A B S T R A C T

Egypt has one of the highest prevalence of hepatitis C in the world. Because therapies have important side effects and high cost, it is important to identify which patients have the best chance to respond to that therapy. Aim of the study is to evaluation of response to pegylated interferon and ribavirin therapy in chronic hepatitis C patients in Upper Egypt and to determine predictors of response to this therapy in those patients. We studied 105 patients who are candidate for peg- INF and ribavirin treatment. Baseline assessment included clinical history, physical examination, body mass index (BMI), hematological (CBC), biochemical (liver function tests, serum creatinine and fasting blood sugar), serological (HBVs Ag and HCV Ab), virological tests (HCV RNA by polymerase chain reaction (PCR), thyroid stimulating hormone (TSH), alpha feto protein (AFP), anti –nuclear antibody (ANA) and liver biopsy . Also, we studied a recent predictor of response (serum 25 hydroxy vitamin D level).We followed the patients for 48 weeks of treatment and 6 months after stoppage of treatment to detect SVR. Patients who achieved SVR were 51.3% of patients. no significant relation between SVR and vitamin D level, age, sex, BMI, histological grading and staging and AFP but there was a significant difference between SVR and virus RNA count by PCR testing ($P < 0.026$). We concluded that HCV viral load could be used as a predictor of response to peg –INF and ribavirin therapy and decision of treatment should be individualized for each patient after corporation of all predictors.

Keywords

Vitamin D, Pegylated Interferon and Ribavirin, Chronic Hepatitis C

Introduction

Hepatitis C virus (HCV) is considered as a global health problem as declared by the World Health Organization with approximately 3%of the world population, roughly 170-200 million people infected (1). In Egypt the situation is quite worse. Egypt has one of the highest prevalence of hepatitis C in the world. According to government figures about 10-15% of the Egyptians are carrying hepatitis C antibodies, Five millions of those are actively infected .About 90 % of them are genotype 4 (2).

The primary goal of HCV therapy is to cure infection which results in eliminating detectable circulating HCV after cessation of treatment. Secondary goals of antiviral therapy include improvement in histology, quality of life and prevention of hepatocellular carcinoma. Sustained virological response (SVR) is defined as an undetectable HCV RNA level 24 weeks after treatment withdrawal. SVR is generally associated with resolution of liver disease in patients without cirrhosis.

Combination therapy using pegylated interferon and ribavirin till recently was the gold standard of treatment for chronic hepatitis C. However, nonresponse to this therapy is both common and associated with several factors (3).

The recommended regimen is INF alfa-2a or *2b weekly injection and ribavirin at 1,000 to 1,200 mg daily for 6 to 12 months.* The decision to initiate antiviral therapy should be made on an individualized basis that considers severity of liver disease, co-morbid conditions, the potential for serious side effects and the likelihood of response.

Because these therapies have important side effects and high cost, it is important to identify which patients have the best chance to respond to that therapy. Accordingly the ability to accurately predict the response of patients to antiviral therapy is of great interest. In general, predictors of response are related to host (genetic, biochemical or histological) and viral factors(1).

In several studies, multivariate analysis of baseline factors have been identified several variables that are associated with greater likelihood of sustained virological response .Viral factors include hepatitis C virus genotype and lower baseline viral level (from 600,000-800,000 IU/ml or less)was shown to be an independent predictor of sustained virological response regardless of genotype in numerous studies (4, 5). Disease related factors include absence of bridging fibrosis or cirrhosis on liver biopsy. The presence of advanced liver fibrosis and cirrhosis has long been recognized to be associated with lower response rates to interferon based therapy (6).

Host factors include age, sex, race, body mass index and alcohol consumption(7). Insulin resistance, IP-10 and IL-B28 are new and important predictors of response to pegylated interferon therapy.

Vitamin D is a potent immune-modulator that favors innate immunity and cell differentiation (DeLuca, 2004 8). Low serum levels of 25-hydroxyvitamin D (< 20 ng / mL) prevent macrophages from initiating this innate immune response, which may explain why African Americans, who are often vitamin D deficient, are more prone to contracting viral infections and tuberculosis than Caucasians are. Moreover, vitamin D improves insulin sensitivity, suppresses pro-inflammatory cytokines, increases anti-inflammatory cytokines, and improves CD4 T cell hyper-responsiveness (9). Vitamin D deficiency is very common (92%) among patients with chronic liver disease, and at least one third of them suffer from severe vitamin D deficiency (< 12 ng /mL) (10).

Recently vitamin D has emerged as a new predictor and studies showed that vitamin D deficiency was association with nonresponse to pegylated interferon and ribavirin therapy (11) and a recent study by Nimer et al ., 2012(12) showed that adding vitamin D to pegylated interferon and ribavirin improves the SVR in genotypes 2 and 3 na□ve patients

Genotype 4 can be singled out as the one least studied among the other genotypes. This is not surprising considering that research is not a priority, to put it mildly, in the Middle East including Egypt. From the previously mentioned studies it seems that there is great controversy regarding the response rate and the predictors of response to pegylated interferon with genotype 4. In addition, it is the least studied genotype in comparison of other genotypes(13).

The aim of this study to evaluate the response to pegylated interferon and ribavirin therapy in chronic hepatitis C patients in Upper Egypt and to determine predictors of response of these patients to this therapy.

Patients and Methods

The present study included 105patients (73 males and 33 females) with chronic HCV infection. Their ages ranged from 20-60 years. All patients were attending the center for treatment of chronic hepatitis C in Assiut in the period between April 2013 and March 2014 every week to receive treatment of chronic HCV in the form of pegylated interferon injection every week and ribavirin tablets every day. Before beginning of treatment there was a period for preliminary evaluation and laboratory tests. Baseline assessment included clinical history, physical examination, body mass index (BMI), as well as routine hematological (CBC), biochemical (liver function tests, serum creatinine and fasting blood sugar) , serological (HBVsAg and HCV Ab) and virological tests (HCV RNA by polymerase chain reaction (PCR) . Other tests include thyroid stimulating hormone (TSH), alpha feto protein (AFP) and anti –nuclear antibody (ANA). Also, we have studied a recent predictor of response (serum 25 hydroxy vitamin D level). It was performed by kits supplied from Orgentec lot number ORG 270 based on enzyme linked immunosorbent assay. Pretreatment liver biopsy for pathological grading and staging was performed in all studied patients .The hepatic necroinflammatory activity and stage of fibrosis in the biopsies was evaluated according to the METAVIR scoring system. Before inclusion in the study, all participants gave informed consents and the study was approved by the local Ethics Committee of Assiut Faculty of Medicine. The inclusion and exclusion criteria were according to the protocol of National Committee for Control of Viral Hepatitis. After an initial assessment, patients were treated with pegylated interferon (40 kD; Pegasys at a dose of 180 µg per week or 12 KD or Pegintron at a dose of 1.5 µ/ kg body weight) plus ribavirin at a dose of 1000-1200 mg daily as per body weight-1000 mg if ≤75 kg and 1200 mg if ≥75 kg-for 48 weeks. Follow up during treatment period (48 weeks) was done during each visit with regular blood picture and liver function testing and to detect any complications of treatment. PCR testing was done at weeks 12,24, 48 and 72 to detect complete early viral response(EVR), End-of-treatment viral response (ETVR) and sustained viral respone (SVR).

All included patients were classified into patient who achieve sustained virological response and patients with non response and analyze their virological responses in relation to host and viral factors such as age, sex, body mass index, liver enzymes, and stage of fibrosis, vitamin D level and HCV PCR concentration at baseline.

Results and Discussion

In this study we enrolled 105 patients with chronic hepatitis C (73 males and 32 females) who fulfilled the study criteria. Finally 72 patients completed the course and were included in the study .They were54

(75%) males and 18 (25%)females. The age of patients ranged from 20-60 years. The rest of patients failed to complete the course (stopped treatment by their selves or developed complications such as anemia, granulocytopia) and the rest of them their data were not complete.

Six months after the end of treatment patients were categorized into two groups according to their viral load status by PCR quantification: Group (1): patients who achieved sustained virological response (SVR). Group (2): patients who didn't achieved sustained virological response (non SVR) including non-response, breakthrough and relapse.

In our study, 37 (51.4%) patients showed SVR and 35 (48.6%) patients showed non SVR. Those with non SVR were divided as 6 (8.3%) patients were non responders to therapy, 19 (26.4%) patients showed breakthrough and 10 (13.9%) patients were relapsers (Fig. 1).

Patients with SVR were higher in younger age group, but no significant relation between SVR and BMI was found. SVR was higher in female group 66.7 % versus 46.3 % in male group. No significant relation between SVR and pretreatment ALT (P value = 0.75, AST level (P value = 0.17) and AFP (P value= 0.64). There is significant relation between SVR and viral load assessed by PCR (P value = 0.026), table 1.

The virological response was assessed in relation to Metavir system where no statistically significant difference in Metavir grades and stages and SVR (P= 0.89 and 0.41 respectively), table 2.

In our study, there was no statistically significant difference between vitamin D levels in those with or without SVR; also, we found no statistically significant difference between vitamin D level and age, sex, BMI, HCV RNA and pretreatment grade and stage of chronic HCV (table 3, 4).

In the present study , SVR was achieved in 51.4 % of treated patients , this percentage was very close to SVR reported by Idrees and Riazuddin (3) which was 50.5 % and Ibrahim et al ., 2014 (14) which was 58.6 %. While, higher response rate (68.6%) was reported by Esmat et al (15). Also, nearly the same response rate (63.3%) was reported by Varghese et al(16) in chronic HCV genotype 4.

The response to INF- α treatment was better in women (66.6%) than men (46.3%).This agrees with that reported by Idrees and Riazuddin(3) who found that the response to INF in women was 56.7% and in men was 47.6%. females may be more compliant with treatment than males.

The relationship between the response to INF-α treatment and age is controversial .In our study there was no statistically significant relation between age and SVR. While, Omran et al(17) and Roeder et al(18) reported that patients who achieved SVR were significantly younger than those with non SVR.

We found no statistically significant relation between the BMI and response to interferon therapy. The same results were reported by Pattullo and his collages (19). In contrary, higher BMI was identified as a significant risk factor of non response to interferon therapy by Gheorghe et al(20). The non-significant relation between BMI and response to interferon therapy in our study could be explained by the narrow variations of BMI in the included patients (limited by the national protocol).

In this study, there are no significant differences in baseline levels of ALT, AST

in patients with or without SVR. These results were similar to that reported by David et al(21) and Antonov et al (22). Mohamed et al(23) found similar results with exception that, there was a significant difference in AST level between responder and non-responder patients.

Table.1 Virological response versus pretreatment ALT, AST, viral load and AFP

	SVR (n= 37)	Non-SVR (n= 35)	P-value
ALT:			0.748
Mean ± SD	56.16 ± 30.76	52.89 ± 23.21	
Median (Range)	50.0 (9.0 – 183.0)	48.0 (17.0 – 101.0)	
AST:			0.171
Mean ± SD	44.70 ± 22.51	49.46 ± 20.15	
Median (Range)	44.0 (14.0 – 130.0)	50.0 (10.0 – 100.0)	
PCR:			0.026*
Mean ± SD	597568.78 ± 826169.02	1468759.03 ± 2856714.09	
Median (Range)	173000 (9100-3130000)	384000 (53000-15400000)	
AFP:			0.636
Mean ± SD	2.86 ± 2.17	3.17 ± 2.43	
Median (Range)	2.5 (0.3 – 9.8)	2.8 (0.4 – 11.1)	

Table.2 Virological response versus grading and staging system

	SVR (n= 37)	Non-SVR (n= 35)	P-value
A:			0.893
A1(no.) % within A1	23 52.3%	21 47.7%	
A2 (no.) % within A2	10 47.6%	11 52.4%	
A3 (no.) % within A3	4 57.1%	3 42.9%	
F:			0.408
F1(no.) % within F1	22 45.8%	26 54.2%	
F2 (no.) % within F2	12 63.2%	7 36.8%	
F3 (no.)	3	2	

% within F3	60.0%	40.0%	

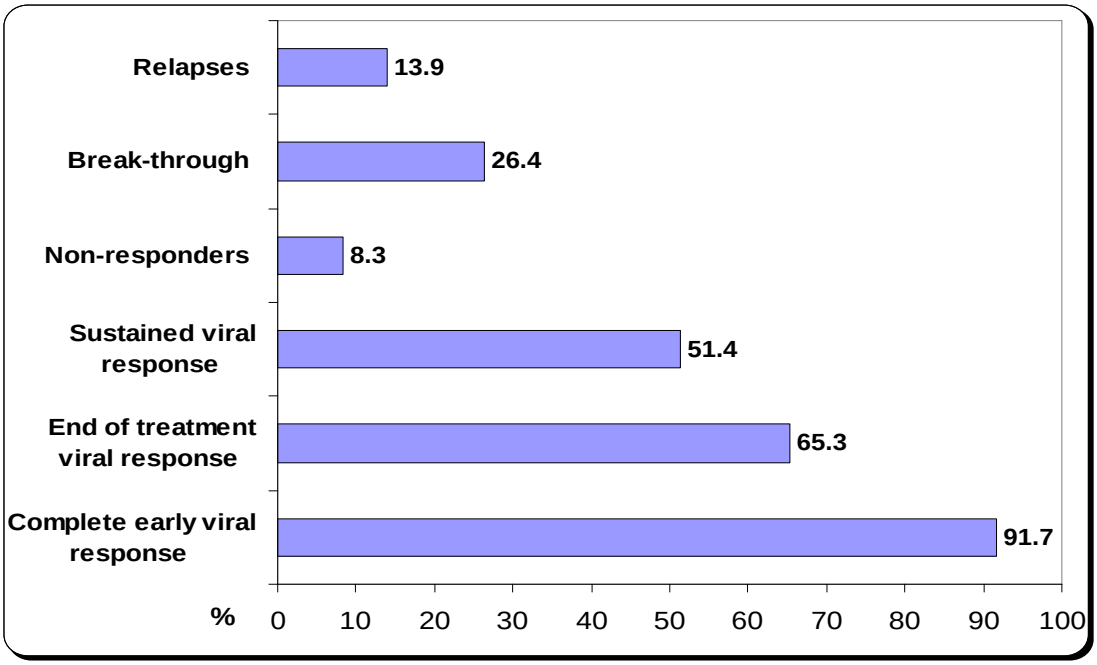
Table.3 Relationship between demographic, viral load and histological variables and baseline serum 25-OH vitamin D level

	Vitamin D level				P-value
	< 32 (n= 44)		≥ 32 (n= 28)		
	No.	%	No.	%	
Age:					0.488
Mean ± SD	38.50 ± 11.35		41.14 ± 12.52		
Range	20.0 – 58.0		21.0 – 60.0		
Sex:					0.577
Male	34	77.3	20	71.4	
Female	10	22.7	8	28.6	
BMI:					0.922
Mean ± SD	25.91 ± 3.59		25.94 ± 2.90		
Range	17.0 – 35.5		22.2 – 30.8		
HCV RNA:					0.335
Mean ± SD	701787.4±887909.9		1522784.5±3163780.3		
Range	9100 - 3850000		40000 - 15400000		
Pretreatment grading:					0.629
A1	25	56.8	19	67.9	
A2	14	31.8	7	25.0	
A3	5	11.4	2	7.1	
Pretreatment staging:					0.505
F1	30	68.2	18	64.3	
F2	10	22.7	9	32.1	
F3	4	9.1	1	3.6	

Table.4 Relation of different treatment responses to vitamin D level in all studied patients

	Vitamin D level				P-value
	< 32 (n= 44)		≥ 32 (n= 28)		
	No.	%	No.	%	
Complete early viral response	41	93.2	25	89.3	0.884
End of treatment viral response	29	65.9	18	64.3	0.888
Sustained viral response	24	54.5	13	46.4	0.502
Non-responders	3	6.8	3	10.7	0.884
Breakthrough	12	27.3	7	25.0	0.831
Relapses	5	11.4	5	17.9	0.669

Figure.1 Variable responses to INF therapy



In our study, there is significant difference in HCV RNA level by PCR in patients with or without SVR. In agree with our results, Hadziyannis et al(24) and Xie et al (25) found that low viral concentration was associated with clearance of the virus. The SVR rate was considerably higher in patients with a pretreatment viral load less than 800.000 IU/ml. Derbala and his collages (2) found lower SVR among genotype 4 infected patients with high viral load.In contrary to our results, Hu et al(26) found SVR among patients with higher viral load.

We found statistically insignificant relation between grade of hepatic inflammation (byMetavir's scoring system) and response to interferon therapy. This is in agreement with Faisal et al(27). This can be explained by the fact that the grade of inflammation correlates with the underlying immune response in the form of increased level of cytokines such as TNF α, INF γ and IL2 which are responsible for hepatic inflammation. In disagreement to our results Lee et al(28) found that SVR is significantly related to Metavir score for grading of chronic HCV.

Beside the above mentioned parameters many studies suggested vitamin D as an additional predictor of SVR, whereas low pretreatment (levels of 25-OH-Vitamin D3 (25(OH) D3)(< 32 ng/ml) is associated with significantly low responsiveness to antiviral therapy (11). However, on the other side, recent findings also indicate that the pretreatment concentration of vitamin D is not always capable of predicting treatment outcome in chronic HCV infection (29).In our study we found no statistically significant relation between vitamin D level in the serum and SVR to interferon therapy. In contrary to our results Petta and his collages (30) have retrospectively analyzed a cohort of 167 patients treated with peg-interferon and ribavirin for hepatitis C, and detected an association between lower vitamin D serum levels and failure to achieve SVR. A study by Nimer et al(12) found the same findings. At study in Egypt by Mohamed and his collages (31) (performed on 50 Egyptian patients) concluded that Vitamin D deficiency predicts an unfavorable response to interferon-based treatment of HCV. In agreement of our results, a recent study by Kitson et al(32) also found that the baseline 25(OH)D level is not associated with SVR to PEG-IFN plus RBV therapy in chronic HCV infection , regardless of genotype and any effect of vitamin D supplementation on SVR is yet to be definitively determined. Our findings are supported by several studies in the literature evaluating vitamin D levels in chronic viral liver disease. Duarte and colleges (33) evaluated 100 persons with chronic hepatitis C and found no difference in mean vitamin D levels in those with and without cirrhosis. In our study we found no statistically significant difference between vitamin D level and age, sex, BMI, HCV RNA level, pretreatment grading and staging of chronic HCV. This is against that reported by Petta et al (30).

In conclusion, SVR to pegylated IFN and ribavirin therapy in Egyptian patients (G4) is about 50 % which is low .This raises the importance of detecting predictors of response to this treatment. Low pretreatment viral load was significantly associated with SVR. Vitamin D level was not significantly associated with SVR. Treatment should be initiated on individualized basis after corporation of all predictors in each patient. Also, we must study vitamin D level and other studied predictors as predictor of response with the new direct acting antiviral drugs (DAADs) in our patients.

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