Original Article

ESTIMATION OF LYMPHOCYTE SUBPOPULATIONS AS PREDICTORS OF RESPONSE TO PEGYLATED-INTERFERON THERAPY IN CHRONIC HEPATITIS C VIRUS INFECTION

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ABSTRACT

Introduction: Hepatitis C virus (HCV) is one of the endemic viral infections in Egypt and there is no vaccine for HCV and the only available treatment, a combination of α-interferon and ribavirin, is effective in a minority of patients, various viral and host factors may affect response to treatment.

Aim of the Work: Is to examine changes in lymphocyte subpopulations in the peripheral blood of chronic hepatitis C patients before and after interferon therapy and to compare between immunological and virologic predictors of treatment outcome.

Patients and Methods: Immunophenotyping of peripheral blood lymphocyte subpopulations of twenty chronic HCV patients were studied by four-color flowcytometry before and three hours after the first interferon injection and at 4 and 12 weeks during therapy. The percentages of total T, T helper, activated T helper, T regulatory, T cytotoxic, activated T cytotoxic, T memory, natural killer and B lymphocytes were evaluated with the use of CD3, CD4, CD8,CD25, CD127, HLA-DR, CD45RO, CD56 and CD19 conjugated monoclonal antibodies, respectively.

Results: No significant difference was found in the ratio of different lymphocyte subpopulations in patients before and three hours after administration of interferon; after 4 weeks and after 12 weeks of treatment, except for CD3CD8 cytotoxic T lymphocytes which showed significant difference between complete responders and non responders (significant decrease in cytotoxic cell ratio after three hours as compared to pretreatment). Conclusions: Although measurement of HCV RNA viral load is the gold standard for assessment of response to therapy, immunophenotyping of cytotoxic T lymphocytes can be used as an immunological predictor of outcome of treatment.

Conclusion: This immunological predictor of outcome of treatment (Cytotoxic T lymphocyte decrease at three hours) is earlier than the virologic predictor (HCV RNA at 12 and 24 weeks) and can thus decrease the risk of prolonged futile interferon therapy, also measurement of HCV RNA viral load at 12 weeks is not sufficient and should repeated at 24 weeks of treatment.

Keywords: *Hepatitis C virus, pegylated-interferon, lymphocyte subsets.*

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INTRODUCTION

Hepatitis C virus (HCV) was identified in 1989 as an important cause of parenterally transmitted hepatitis *(Wedemeyer, 2007)*. Chronic HCV infection is a major cause of liver disease and the leading indication for liver transplantation worldwide. About 150 million people are chronically infected with hepatitis C virus and more than 350,000 people die every year from hepatitis C-related liver diseases *(WHO 2012)*. Egypt has possibly the highest HCV prevalence in the world; 10%–20% of the general populations are infected and HCV is the leading cause of hepatocellular carcinoma and chronic liver disease in the country *(Habib et al. 2001).*

HCV is a spherical, enveloped, single-stranded RNA virus belonging to the Flaviviridae family. RNA-dependent RNA polymerase lacks proofreading capabilities and generates a large number of mutant viruses known as quasispecies. Six major HCV genotypes and numerous subtypes have been identified *(De Bruijne et al. 2009)*, approximately 90% of Egyptian HCV isolates belong to a single subtype - 4a, which responds less successfully to interferon therapy than other subtypes *(Miller FD and Abu-Raddad LJ, 2010).*

There is no vaccine for HCV and the only available treatment, a combination of α-interferon and ribavirin, is effective in only a minority of patients, with an eradication rate and long-term disease remission in approximately 40% of patients. This treatment regimen lasts for 24 or 48 weeks and requires parenteral therapy that is costly and has frequent side-effects *(Zaky et al. 2011)*. Ultimately, up to 10% of patients cannot complete a full course of therapy secondary to debilitating side effects.

Modification of the pharmacokinetic profile of IFN α-2a through pegylation has resulted in a marked improvement of drug efficacy for the treatment of HCV infection. The in vitro and in vivo properties of pegylated- interferon are similar to those of other, recombinant type I interferons. Specifically, PEG-IFN can induce intracellular antiviral activity, inhibit the proliferation of several tumor cell lines, activate natural killer cell-mediated tumor cytolysis, induce cytokine synthesis and release by immune effector cells similarly to other interferon preparations. Other advantages include longer half-life, more reduced distribution and lower elimination rate than the respective non- pegylated IFN α *(Krawitt EL et al. 2006)*. There is an increased efficacy seen in patients with HCV infection treated with PEG-IFN, higher sustained virologic response rates in patients with chronic hepatitis C have been reported for pegylated- interferon compared with standard interferon both in monotherapy as well as in combination therapy with ribavirin *(Zaky et al. 2011)*.

Different patients respond differently to this α-interferon treatment regimen as various viral and host related factors are responsible for such diversity in responses. HCV genotype and the pre-treatment viral load influence the antiviral response rates. Among host related factors, age and sex are very important as there is a decrease the risk of disease progression with younger age and female patients *(Akram et al. 2011)*.

Viral kinetics, such as rapid viral response (RVR) and early virologic response (EVR) are new and more accurate predictive tools. However, the use of viral kinetics is limited by the fact that therapy must be taken for up to 12 weeks before prognosis can be accurately determined *(Martinez-Camacho et al. 2011)*.

Immunological factors play a prominent role in controlling HCV infection, whose eradication requires co-ordinated inter-play between innate and adaptive immunity. A vigorous and multi-specific immune response, involving CD4 and CD8 T lymphocytes, NK cells, B lymphocytes and dendritic cells (DC), is associated with viral clearance and disease resolution *(Ferri et al. 2011)*. In chronic hepatitis C, multifaceted immune dysfunction may be implicated in the persistence of HCV including dendritic cells, NK cells and T cells *(Rehermann 2009)*.

Some investigators have reported that the dynamics of immune cells throughout the therapy are involved in the efficacy of PEG-IFNα and ribavirin. In chronic HCV infection, the enhancement of HCV-specific Th1 response or dendritic cell function has been reported to be involved in therapeutic HCV eradication *(Kamal et al. 2002; Pachiadakis et al. 2009)*.

AIM OF THE WORK

Is to examine changes in lymphocyte subpopulations in the peripheral blood of patients with chronic hepatitis C before and after interferon therapy, to correlate between these changes and response to treatment and to compare between immunophenotyping of lymphocytes as an immunological predictor of outcome of treatment and the viral load as a virologic predictor.

PATIENTS AND METHODS

Patients: This study was conducted on twenty patients (18 males and 2 females) with chronic hepatitis C virus infection admitted to the Interferon Unit in The Cardiac and Digestive System Diseases Center, Sohag, Egypt during the period from July 2012 to July 2013. Their ages ranged between 20 to 60 years. The study protocol was approved by the local ethics committee and all patients gave their consent prior to the study.

Inclusion Criteria: Enrolled patients were;

1. Serologically positive for antibodies to HCV by third generation ELISA and HCV RNA positive by reverse transcription-polymerase chain reaction(RT-PCR).
2. Histologically proved as chronic hepatitis grade 2 or more according to Metavir scoring system
3. Elevated serum alanine aminotransferase (ALT) above 40 IU/L.

Exclusion Criteria:

1. Patients serologically positive for hepatitis B surface antigen (HBsAg) and/ or antinuclear antibodies.
2. Patients with decompensated, alcoholic or metabolic liver diseases.

Methods:

(I)Virologic Studies: Blood was collected for serum separation from all patients; 10 ml of venous

blood was obtained by venipuncture from each patient. The blood was left to coagulate at 37°C for 45 minutes and blood was then centrifuged at 3000 rpm for 10 minutes. The collected serum was then aliquoted into 4 cryotubes and stored at -20°C until examination.

a. **Detection of anti-HCV AB by ELISA:** Antibodies to HCV were detected with ELISA version 3.0 (Sorin Biomedica, Italy) according to manufacturer's instructions.

b. **HCV RT-PCR:** RNA was extracted from patients' sera according to manufacturer's instructions by Qiagen (Germany). The RT-PCR was performed as described by *Boom et al. (1990)*.

(II) INF Treatment Regimen: Patients were injected subcutaneously with 180 µg of Pegylated IFN-α2a (PEGASYS®; Roche, Basel, Switzerland) once per week for 48 weeks in combination with weight based ribavirin (Ribavirin® or Rebetol®) in a daily dose of 11 mg/kg.

The response to IFN therapy was evaluated at 12, 24 and 48 weeks, according to the PCR results (viral load) and patients were classified into two groups:

Group I (Complete Responders): Defined as patients who clear viral RNA by the end of treatment at 48 weeks.

Group II (Non-Responders): Defined as patients who fail to clear viral RNA at 12, 24 or 48 weeks.

A rapid viral response (RVR) is defined as a qualitative undetectable HCV-RNA at week 4 of treatment (<50 IU/ml) whereas an early virologic response (EVR) is defined as achieving either a ≥2 log viral load decrease from baseline or a qualitative undetectable (<50 IU/ml) HCV-RNA at week 12 after initiation of treatment. A sustained virologic response (SVR) was defined as a qualitative undetectable HCV viral load (<50 IU/ml) 24 weeks after discontinuation of therapy.

(III) Immunophenotyping of Peripheral Blood Lymphocytes

Peripheral Blood Lymphocytes Processing: Fresh 2 ml ethylene diamine tetraacetic acid-anticoagulated peripheral blood samples were collected before treatment, three hours after first injection and at 4 weeks and 12 weeks during therapy. According to manufacturer's instructions, 10 µl MoAb was added to 100 µl EDTA blood then vortexed and incubated for 15 min at room temperature in the dark, then 1ml lysing reagent (VersalyseTM -Beckman Coulter) was added and immediately vortexed then incubated for 10 min. at room temperature in the dark. We verified that there were no significant differences in the investigated marker levels in samples stained immediately after collection in comparison with samples stained up to a maximum of 24-hours time interval post sampling and stored at 4°C.

Fluorochrome-conjugated monoclonal antibodies (MoAbs) were used for staining lymphocytes namely anti-CD4, anti-HLA-DR labeled with fluorescein isothiocyanate (FITC), anti-CD8, anti-CD25, anti-CD56 labeled with phycoerythrin (PE), anti-CD3, anti-CD19, anti-CD45RO labeled with electron coupled dye (ECD) and anti CD127 labeled with phycoerythrin-cyanin5 (PE– Cy™5) (Beckman Coulter, France).

Lymphocyte Subpopulation Assays: The Lymphocyte subpopulations analysed were CD3/CD4 (Total. T helper cells), CD4/CD25 (activated T helper cells), CD4/CD25/CD127low (T regulatory-Tregcells), CD3/CD8(total T cytotoxic cells), CD8/HLA-DR (activated T cytotoxic cells), CD4/CD45RO and CD8/CD45RO (memory T cells), CD3- CD56+ (natural. killer cells), CD19 (B lymphocytes). The blood samples were analysed with Epics-XL flow cytometer (Coulter, USA). Lymphocytes were analysed using a gate set on forward scatter versus side scatter, with a standard 4-color filter configuration using System II software version 3.0 (Coulter, USA).

We recognized two different strategies of Treg identification based on surface markers:

1. Gating on CD4 cells with high expression of CD25.
2. Gating on CD4 cells expressing CD127 low/negative levels (CD127low) cells.

Statistical Analysis:

Values were presented as percentages or mean± standard deviation. Fisher exact test was used for categorical data and non parametric test was used for quantitative data. Variables with uneven distribution were analyzed with the Mann-Whitney's U test to compare between the two groups. For all tests P value < 0.05 was considered significant.

RESULTS

Patients' Demographics: Patients' demographics according to treatment outcome (non responders and complete responders) were summarized in Table (1). Complete responders were defined as clearance of HCV RNA at the end of treatment.

Table 1: Patients' demographics: Comparison between non responder (NR) and complete responder (CR) groups.

	NR		CR		P-value
	No	%	No	%	
Sex					
Male	7	87.5	11	91.7	0.04
Female	1	12.5	1	8.3	
Liver histology (Metavir score)					
≤ A2F2	6	75	10	83.3	0.3
> A2F2	2	25	2	16.7	
Serum HCV RNA at 12 weeks					
Negative	3	37.5	12	100	0.005
Decrease	3	37.5	0	0	
Unchanged	2	25	0	0	
Pretreatment serum AL.T (IU/L)					
< 70	3	37.5	7	58.3	0.6
≥ 70	5	62.5	5	41.7	
Age					
20-29 y	1	12.5	5	41.7	0.2
30-39 y	2	25	2	16.7	
40-49 y	2	25	4	33.3	
50-59 y	3	37.5	1	8.3	
Pretreatment viral. load (copies/ ml)					
<100000	3	37.5	5	41.7	0.4
100000-1000000	2	25	4	33.3	
1000000 >	3	37.5	3	25	

Data were expressed as number or percentage. Abbreviation: NR: Non Responder, CR: Complete Responder.

Results of Peripheral Blood Lymphocytes Immunophenotyping: Flow cytometric analysis of the peripheral lymphocyte subpopulations was done pretreatment, three hours after the first dose of interferon and at 4weeks and 12 weeks of treatment. These results were compared in complete responders and non responders and shown in Table (2).

As shown in Table (3), no significant difference was found in the ratio of different lymphocyte subpopulations in patients before and three hours after administration of interferon, before and at 4 weeks, before and at 12 weeks of treatment, except for CD3CD8 cytotoxic T lymphocytes which showed significant difference between complete responders and non responders (after three hour: pretreatment) (P value > 0.01) (Figure 1). Cytotoxic T lymphocytes decrease significantly in patients who cleared HCV RNA (complete responders) compared with non responders.

Predictive factors affecting the treatment outcome: To clarify the factors that affect the treatment outcome, monovariable analysis was performed (Table 4).

As shown in Table (4), among all factors affecting treatment outcome, HCV RNA clearance at 12 and 24 weeks (virologic predictor) are the most significant predictive factors that can be used (P value > 0.01) predicting the outcome of treatment .

HCV RNA levels of three patients (37.5%) in the non responder group were negative at 12 weeks and reverted to positive at 24 weeks of treatment, (Figures 2a,b), so the assessment of treatment outcome using HCV RNA viral load as virologic

predictor at 12 weeks is not sufficient and should be re-evaluated at 24 weeks of treatment.

Two significant factors were detected that can be used as predictors of outcome of treatment:

1. Immunophenotyping of cytotoxic T lymphocytes at three hours as immunological predictor.
2. HCV RNA clearance at 12 and 24 weeks as virologic predictor.

The immunological predictor of outcome of treatment (Cytotoxic T lymphocytes at three hours) is much earlier than the virologic predictor (HCV RNA at 12 and 24 weeks). As an early predictor, it can assist in decision taking of continuation or discontinuation of therapy and alleviate the high cost and the debilitating side effects of ineffective therapy.

Table 2: Peripheral blood lymphocyte subpopulations in patients before, 3 hours after administration of interferon, at 4 weeks and at 12 weeks of treatment.

Lymphocyte subpopulations	Pretreatment		After 3 hours		4 weeks		12 weeks	
	NR	CR	NR	CR	NR	CR	NR	CR
CD3/CD4	31.8 ±16.	34.5±8.5	31.4±18.4	37.3±8.6	31.1±12.8	34.4±10.4	30.1±15.6	34±10.7
CD4/CD25/ CD127	5.1±1.9	6.2±5.1	4.2±0.6	3.5±1.5	4.8±1.8	4.2±1.5	3.6±1.3	3.4±1.5
CD4/CDRO	25.8 ±13.4	24.1±7.7	23.2±17.9	25.4±8.8	22.1±11.9	22.2±9.1	20.8±14.2	22.1±9.2
CD3/CD8	15±9.5	12.4±5.5	17.3±9.3	21±5.6	16.4±10.1	22.8±8.1	12.2±7	19.7±7.1
CD8/HLA-DR	20.1±12.9	15.6±8	17.1±10.3	13.1±8.6	14.2±13.7	11.7±6.7	10.1±5.1	8.5±5.3
CD8/CDRO	5.5±4.6	5.5±1.9	5.3±5.8	5.3±2.1	3.1±1.1	6.1±4.9	2.6±2.0	3.6±1.7
CD19	9.1±5.3	9.8±6.3	11.3±5.4	12.3±6.4	6.9±3.3	6.7±4.4	5.7±4	5±2.5
CD3-/ CD56	8.1±4.4	10.9±4.5	7.2±4.7	9.2±7.4	9.2±8.5	11.1±5.9	6.2±4	10.3±4.8

Data were expressed as mean ±standard deviation.

Table 3: Ratio of peripheral blood lymphocyte subpopulations in patients before and 3 hours after administration of interferon, before and at 4 weeks, before and at 12 weeks of treatment.

Lymphocyte subpopulation	3 hours: pretreatment			4 weeks: pretreatment			12weeks: pretreatment		
	NR	CR	P value (CR/ NR)	NR	CR	P value (CR/ NR)	NR	CR	P value (CR/NR)
CD3/ CD4	1.06±0.3	1.1±0.2	0.7	1.3±0.8	1±0.3	0.2	1.07±0.3	0.9±0.2	0.5
CD4/ CD25 CD127	0.94±0.4	0.82±0.4	0.6	1.03±0.4	1±0.6	0.9	0.79±03	0.8±0.5	0.9
CD4/ CDRO	1.01±0.5	1.1±0.3	0.6	1.12±0.5	0.8±0.3	0.3	0.6±0.4	0.9±0.2	0.1
CD3/ CD8	1.3±0.4	0.9±0.2	0.01	1.3±0.6	1.1±0.4	0.3	0.8±0.2	0.9±0.2	0.7
CD8/ HLA-DR	0.88±0.2	0.83±0.2	0.6	0.6±0.2	0.7±0.2	0.3	0.6±0.4	0.5±0.2	0.4
CD8/ CDRO	0.93±0.5	1.04±0.3	0.6	0.5±0.3	1.1±0.5	0.1	0.5±0.1	0.6±0.2	0.3
CD19	1.6±0.68	1.4±0.8	0.6	1.7±2	0.9±0.7	0.2	0.7±0.4	0.6±0.4	0.6
CD3-/ CD56	1.16±0.7	0.8±0.3	0.2	2.2±2.7	1±0.5	0.1	0.6±0.3	0.9±0.4	0.1

Data were expressed as mean ±standard deviation.

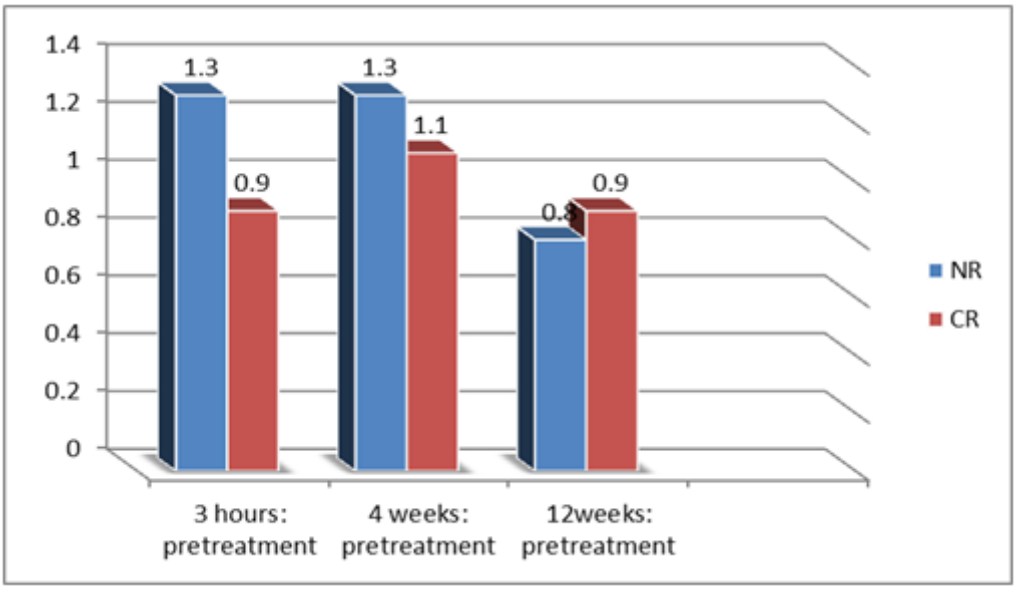

Figure 1: Ratio of cytotoxic T lymphocytes decreases significantly in complete responders when compared with non responders after 3 hour: pretreatment (P value > 0.01).

Table 4: Factors affecting treatment outcome.

Demographic data	NR		CR		P value
	No.	%	No.	%	
Sex					
Male	7	87.5	11	91.7	0.04
Female	1	12.5	1	8.3	
Serum HCV RNA(Pretreatment)					
< 500 000	4	50	7	58.3	0.1
≥500 000	4	50	5	41.7	
Serum HCV RNA (12 weeks)					
Negative	3	37.5	12	100	0.005
Decrease	3	37.5	0	0	
Unchanged	2	25	0	0	
Serum HCV RNA (24 weeks)					
Negative	0	0	12	100	0.001
Positive	8	100	0	0	
Pretreatment T regulatory cells					
≤ 7 %	6	75	10	83.3	0.7
> 7%	2	25	2	16.7	

Data were expressed as number or percentage, Cut off for T regulatory cell is 7 %.

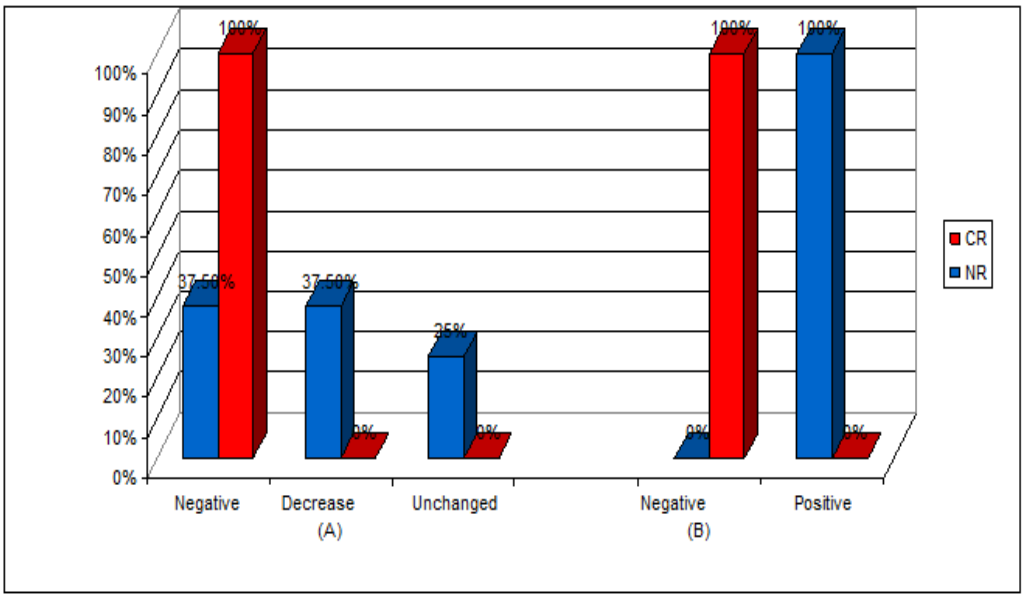

Figure 2: HCV RNA levels (A) at 12 weeks and (B) at 24 weeks of treatment.

DISCUSSION

Cellularimmunity to HCV plays a critically important role in the control and spontaneous eradication of infection. The induction and maintenance of effective antiviral immunity requires functional antigen-specific CD4+ T cells *(Younes et al. 2007)*.

It has been suggested that cellular immune responses, modulated by pegylated IFN-α ribavirin

therapy, play a role in forced viral eradication, based on the immunological properties attributed to these anti-viral compounds *(Zeuzem et al. 2006)*. However, the role of HCV-specific T-cells before and during pegylated IFN-α ribavirin therapy is still controversial *(Ishii and Kozie, 2008)*.

Some studies have shown that achievement of a SVR is associated with high baseline CD4+ and/ or CD8+ specific T-cell responses *(Caetano et al. 2008)*, while others have seen no such relationship *(Barnes et al. 2009)*. Similarly, contradictory results have been reported on the role of HCV-specific T-cells during pegylated IFN-α / ribavirin therapy showing either augmentation (Kamal. et al. 2002) or decline of HCV-specific T-cells in relation to SVR *(Pilli et al. 2007)*.

In this study we examined peripheral blood lymphocytes from 20 patients receiving pegylated IFN-α / ribavirin therapy. All patients were assessed before treatment, after three hours of initial dose, at 4 weeks and at 12 weeks of treatment. The peripheral blood lymphocytes examined were T helper, activated T helper, T regs, T cytotoxic, activated T cytotoxic, memory T, B lymphocytes and natural killer cell.

No correlation was found between changes in peripheral blood lymphocytes and the outcome of pegylated IFN-α ribavirin therapy except for CD3 CD8 cells, which decrease significantly in the responder group after three hours of initial dose while CD3 CD8 cells did not decrease in non responder group except after 12 weeks of treatment.

The cytotoxic T cell response to viral antigens is important for hepatocyte destruction and elimination of HCV via cytolysis of HCV-infected hepatocytes *(Kaneko et al. 1996)*. HLA-DR expression is necessary for this cytotoxicity *(Miyaguchi et al. 1997)*. In a study of five healthcare workers with acute HCV infection post-needle stick injury, the first CD8+ T cells to appear in the blood of the subject who cleared infection were CD38+, reflecting an activated status. The appearance of these cells coincided with the appearance of liver disease, but the cells did not produce IFN-γ. Later in the disease course, disappearance of CD38 positivity coincided with detection of IFN- γ production, viral clearance and resolution of hepatitis *(Thimme et al. 2001)*. Both in vitro and in vivo evidence indicates that interferon stimulation gene induction by IFN- γ inhibits HCV replication *(Lanford et al. 2003)*.

Lancaster et al. found no difference in the number of HCV-specific IFN- γ producing CD8+ T cells as determined by ELISpot through a cross-sectional study of seven subjects with resolved infection and 14 with chronic infection *(Lancaster et al. 2002)*. However Cox et al. reported that HCV-specific CD8+ T cells are more common in patients with chronic HCV infection than in those who have recovered from infection *(Cox et al. 2005)*, raising the suggestion that ongoing antigenic stimulation is necessary to maintain CD8+ T cells.

The kinetics of development of the CD8+ T cell response also appears to be important. For example, Major et al. in a chimpanzee study found that the animals that cleared the infection had an earlier intra-hepatic IFN-γ response than those that developed persistent infection *(Major et al. 2004)*.

Khanna et al. *(2003)* found that in primary herpes simplex virus infection, activated antigen-specific CD8+T cells can be detected as early as six hours after infection *(Khanna et al. 2003)* but *Danijela et al. (2012)* found that this takes 7-12 weeks in HCV infection *(Danijela et al. 2012)*. This delay in the immune response may reflect the fact that HCV infection is primarily localized to the liver, thus HCV antigens may not be available to professional antigen-presenting cells until the infection is relatively well established.

The rapid evolution of the viral quasi-species may outpace the generation of the CD4+ and CD8+ T cell responses and the changes in lymphocyte subpopulations in peripheral blood after interferon therapy could be the result of lymphocyte being trapped at the major inflammatory site, the liver.

In this study, no significant difference could be detected in the proportion of Treg cells in the peripheral blood of responder and non responder at any time during therapy.

CD4+CD25+T cells comprise 2–5% of CD4+T cells in the peripheral blood and contain a sub-population with the capacity to suppress the proliferation of both CD4+ and CD8+ cells, termed regulatory T cells (Treg) *(von Herrath and Harrison, 2003)*. The Treg sub-population is also marked by the intracellular presence of the forkhead transcription factor 3 (foxp3), which is responsible for the development and suppressive function of Treg cells *(Hori et al. 2003)* and by low level expression of the interleukin-7 receptor, CD127 *(Seddiki et al. 2006)*.

Cabrera et al. *(2004)* in a cross sectional study found a higher proportion of CD4+CD25+ cells in peripheral blood of 30 subjects with chronic infection, compared to 15 subjects who had previously cleared infection; these latter subjects also had a lower proportion of Treg than healthy control subjects *(Cabrera et al. 2004)*.

However, Smyk-Pearson et al. *(2008)* found evidence against the role of Treg cells in promoting the development of chronic HCV infection. They reported in a prospective study of 27 acutely infected subjects that there was no significant difference in the proportion of CD4+CD25Hi T cells in the peripheral blood at baseline between the 15 subjects who developed chronic infection and the 12 that subsequently cleared the infection *(Smyk-Pearson et al. 2008)*.

The frequency for both groups was higher than in healthy controls and did not vary over time.

Measurement of HCV RNA viral load is the gold standard for assessment of response to intereferon therapy, but the optimal follow-up time to determine the sustained virologic response in patients with chronic hepatitis C receiving pegylated-interferon and ribavirin is variable.

In our study we found that the assessment of treatment outcome using HCV RNA levels as virologic predictor at 12 weeks is not enough as false negative results were obtained in 37.5% of patients in the non responder group, so HCV RNA levels should be re-evaluated at 24 weeks of treatment.

This is in agreement with Namikawa M et al. who found that the assessment of serum HCV-RNA 12 weeks post treatment, using the highly sensitive real-time PCR assay, is almost as effective as 24 weeks to predict SVR. However, there are false negatives in female patients with a high viral load of genotype Ib when the SVR is predicted by 12 weeks so the current standard with 24 weeks is reasonable, nevertheless assessment of serum HCV-RNA 12 weeks may be effective in most patients *(Namikawa et al. 2012)*. In 2012 Campos-Varela assessed 162 HCV patients with liver transplants receiving treatment with pegylated interferon and ribavirin, and found that HCV-RNA testing to assess SVR at W+12 posttreatment seems as valid as W+24 testing and could be considered for predicting SVR. *(Campos-Varela et al. 2012)*.

CONCLUSION

In our study the significant predictive factors that proved to affect the treatment outcome were immunophenotyping of cytotoxic T lymphocytes at three hours as an immunological predictor and HCV RNA clearance at 12 and 24 weeks as virologic predictor, however immunophenotyping of peripheral blood lymphocytes (Immunological predictor) is much earlier than RNA clearance at 12 and 24 weeks as virologic predictor. We recommend that treatment outcome should be evaluated by immunophenotyping of cytotoxic T lymphocytes at three hours, which is a much earlier marker than HCV RNA levels at 12 weeks, also we recommend that assessment using HCV RNA levels as virologic predictor at 24 weeks of treatment as measurement at 12 weeks is not sufficient.

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ملخص البحث

استخدام الخلايا اللمفاوية للتنبؤ بنتيجة علاج التهاب الكبد الوبائي المزمن (ج) بالإنترفيرون ممتد المفعول

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المقدمة: الفيروس الكبدى (ج) هو احد اسباب العدوى الفيروسية المتوطنة فى مصر و لا يوجد لقاح ضد الفيروس الكبدى (ج)، والعلاج الوحيد المتاح (مزيج من الانترفيرون ألفا وريبافيرين) فعال فى عدد قليل من المرضى و العديد من الاسباب المتعلقة بالفيروس و العائل ربما تؤثر فى الاستجابة للعلاج.

هدف الدراسة: هو دراسة النمط الظاهري لمجموعات الخلايا الليمفاوية في الدم لدى المرضى الذين يعانون من التهاب الكبد الوبائي المزمن (ج) قبل وبعد العلاج بالانترفيرون و المقارنة بين مؤشرات التنبؤ المناعية و الفيروسية لنتيجة العلاج.

المرضى و طرق الدراسة: فحص عشرين مريض يعانون من التهاب الكبد الوبائي المزمن (ج) لتحديد النمط الظاهرى للخلايا الليمفاوية لهؤلاء المرضى باسخدام جهاز التدفق الخلوى رباعى الالوان قبل بدء العلاج و بعد ٣ ساعات من بداية العلاج بالانترفيرون و بعد ٤ و ١٢ اسبوع اثناء العلاج . تم حساب النسب المئوية للخلايا الليمفاوية التائية الكلية و التائية المساعدة و التائية المنظمة و التائية السامة و التائية الذاكرة و الليمفاوية القاتلة الطبيعية و الليمفاوية البائية عن طريق استخدام الاجسام المضادة المصبوغة بالصبغات الفلورسنتية ضد العديد من الانتيجينات الخاصة بالخلايا الليمفاوية.

النتائج: لايوجد فرق هام احصائيا بين نسب الخلايا الليمفاوية المختلفة قبل و بعد ٣ ساعات من بداية العلاج بالانترفيرون و بعد ٤ و ١٢ اسبوع اثناء العلاج , الا فقط فى نسبة الخلايا الليمفاوية السامة و التى اظهرت فرقا هام احصائيا بين المرضى المستجيبين و المرضى الغير مستجيبين للعلاج (فرق واضح بين نسب الخلايا الليمفاوية السامة بعد ٣ ساعات من العلاج مقارنة بقبل العلاج).

الاستنتاجات: على الرغم من ان قياس عدد فيروسات الكبد الوبائي المزمن (ج) هو المعيار الذهبى لتقييم الاستجابة للعلاج. الا انة دراسة النمط الظاهري للخلايا الليمفاوية السامة في الدم يمكن استخدامة كمؤشر تنبؤ مناعى لنتائج العلاج و هذا المؤشر التنبؤى المناعى (نسبة الخلايا الليمفاوية السامة ٣ ساعات بعد العلاج) يعتبر مؤشر مبكر جدا زمنيا عن المؤشرات الفيروسية (قياس عدد فيروسات الكبد الوبائي المزمن (ج) عند ١٢ و ٢٤ اسبوع من العلاج) و بالتالى يقلل من خطورة العلاج الطويل المدى الغير مجدى للانترفيرون و ايضا قياس عدد فيروسات الكبد الوبائي المزمن (ج) عن ١٢ اسبوع هو غير كافى و يجب ان يكرر عند ٢٤ اسبوع من بدء العلاج.