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ORIGINAL ARTICLE

The Role of Hepatic Progenitor Cells (HPCs) and the Predictors of Sustained Virological Response (SVR) to Interferon Therapy in Chronic Hepatitis C Infected Patients

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ABSTRACT

Background: Studying the predictors of SVR to pegylated interferon (PEG-INF) alfa-2a and ribavirin (RBV) therapy in chronic hepatitis C-infected patients is crucial for selecting those who would benefit from therapy. Increasing HPCs in hepatitis C-infected patients were shown to be correlated with increased fibrosis and response to therapy. HPCs could be detected in the liver by immunohistochemical expressions of cytokeratin (CK) 7 and CK19.

Aim of the Work: 1. Evaluate the response rate to interferon based treatment in chronic hepatitis C-infected patients. 2. Detect the predictors of SVR to treatment. 3. Study the correlations between CK7 and CK19 expressions and treatment response.

Patients and Methods: This study included 483 chronic hepatitis C-infected patients who fulfilled the study criteria and underwent clinical, biochemical and virological assessments before treatment and at 12, 24, 48 and 72 weeks post-treatment. Only 330 patients completed the course and were included in the statistical analysis. Only 50 specimens were examined for CK7 and CK19 expression using avidin, biotin, peroxidase technique.

Results: SVR was achieved in 132/330 (40%) of patients. SVR was significantly higher in females ($P<0.01$), younger age group ($P<0.004$) and patients who had lower body mass index; BMI ($P<0.001$). There was significant inverse relation between SVR and aspartate aminotransferase (AST) and alpha feto-protein (AFP); $P<0.000$ and <0.000, respectively. The independent predictors of SVR were younger age and lower AST ($P<0.02$ and <0.02, respectively). There was significant association between CK7 and/or CK19 expressions and grade of necro-inflammation ($P<0.033$ and <0.026 respectively) and/or advanced stage of fibrosis ($P<0.001$ and <0.000 respectively). There were significant inverse relations between SVR and the stage of hepatic fibrosis ($P<0.001$) and CK19 expression ($P<0.000$).

Conclusion: Forty % of patients with chronic hepatitis C who completed the course of combination therapy achieved SVR. Younger age and lower pre-treatment AST are independent predictors of SVR. In chronic hepatitis C, progenitor cell activation is correlated with the grade and stage of disease. Proliferating HPCs as assessed by CK7 and CK19 expressions may play a role in hepatic regeneration occurring in this setting and could be incorporated in assessment of treatment response of patients with HCV infection.

Key Words: Hepatitis C virus (HCV), hepatic progenitor cells (HPCs), end of treatment virologic response (ETVR), sustained virologic response (SVR), cytokeratin; CK7 and CK19.

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ABBREVIATIONS

Hepatic progenitor cells (HPCs), sustained virologic response (SVR), pegylated interferon (PEG-INF) alfa-2a and ribavirin (RBV), cytokeratins (CK), end of treatment virologic response (ETVR), aspartate aminotransferase (AST), alpha feto-protein (AFP), alpha feto-protein (AFP), hepatitis C virus (HCV), alanine aminotransferase (ALT), body mass index (BMI), thyroid stimulating hormone (TSH) level, complete blood count (CBC), polymerase chain reaction (PCR), hematoxylin and eosin (H&E), phosphate buffered saline (PBS), early virologic response (EVR).

INTRODUCTION

Hepatitis C virus (HCV) infection is a serious global health threat. About 170 million people are infected worldwide. Despite considerable reduction of the incidence of new infections, the prevalence of HCV is predicted to remain constant in the near future *(Lawson et al. 2007)*. In Egypt the situation is quite worse. Egypt has the highest prevalence of hepatitis C in the world, the national prevalence rate of HCV antibody positivity has been estimated to be between 10-13% *(Mohamed, 2004)*.

The current combination therapy of chronic HCV consisting of PEG-INF plus RBV for at least 24-48 weeks may be accompanied by numerous potentially dose limiting side effects and SVR rates are still unsatisfactory with only approximately 50% in genotype1 *(Manns et al. 2001)*. In HCV, the primary therapeutic goal is SVR, defined as undetectable HCV RNA at the end of a 24-weeks follow up period after treatment completion *(Fried et al. 2002)*. Treatment may reverse or delay the progression to cirrhosis and further development of hepatocellular carcinoma *(Hughes and Shafran, 2006)*.

Treatment response in patients who have chronic HCV infection is quite heterogeneous and variably depends in different studies on host factors as age, sex, alanine aminotransferase (ALT) levels, body mass index (BMI) and liver fibrosis as well as viral factors, such as serum concentration of HCV RNA at the time of initiation of antiviral therapy and HCV genotypes *(Berg et al. 2003)*. However, to the best of our knowledge, predictors of treatment outcome have not been evaluated in patients with HCV infection in Upper Egypt.

HPCs are small periportal cells that are bipotential and capable of proliferation and differentiation into hepatocytes and bile ductular epithelium. Activation of these cells is histologically detectable only when hepatocyte proliferation is suppressed *(Knight et al., 2005)*. Experimental studies in rodents suggest that activation of oval cells that are equivalent to HPCs in human- could be the mechanism by which liver may replace destroyed parenchyma in chronic liver disease *(Evarts et al. 1987)*.

The presence of advanced liver fibrosis and cirrhosis has long been recognized to be associated with lower response rate to interferon based treatment *(Poynard, 2000)*. It was shown that increased HPCs in HCV-infected patients correlated with increasing fibrosis *(Tan and Park, 2002)*. HPCs could be detected in the liver by using monoclonal antibodies for CK7, 8, 18 and 19, as well as to 'hepatocyte specific' antigen; HepPar1 by a standard avidin-streptavidin complex technique *(Hsu and Raine, 1981)*.

AIM OF THE WORK

1. Evaluate the response rate to interferon based treatment in chronic HCV patients.
2. Detect the predictors of sustained response to treatment.

3. Study the correlation between the HPCs as assessed by CK7 and/or CK19 expressions and treatment response in those patients.

PATIENTS AND METHODS

This study included 500 patients (279 males, 221 females) with chronic HCV infection. Their ages ranged between 19-58 years referred to the Department of Tropical Medicine and Gastroenterology, Sohag University Hospital for liver biopsy as a pretreatment requisite for starting PEG-INF and RBV therapy in the period from January (2010) to January (2011). Before inclusion in the study, all participants gave an informed consent and the study was approved by the Local Ethics Committee.

Inclusion Criteria: According to the recommendations of the National Committee for Control of Viral Hepatitis inclusion criteria comprised: Patients with positive anti-HCV and HCV RNA for more than 6 months and negative HBsAg, white blood cell count >4000/mm^3, neutrophil count >2000/mm^3, platelets >100,000/mm^3, hemoglobin >12 gm/dl for females and 13 gm/dl for males, prothrombin time within normal range, direct bilirubin <0.3 mg/dl, indirect bilirubin <0.8 mg/dl, fasting blood sugar within normal range, serum albumin >3.5 gm/dl, stage I if ALT is high, stage II viral hepatitis and stage III patients who are upper endoscopically negative for esophageal varices, normal serum creatinine, normal thyroid stimulating hormone (TSH) level, AFP <100 ng/ml, female patient and male patient's wife practicing adequate contraception, BMI <30.

Exclusion Criteria: According to the recommendations of the National Committee for Control of Viral Hepatitis; patients were excluded if they have liver disease other than HCV (by liver biopsy), co-infection with HBV or human immunodeficiency virus, autoimmune disease, advanced liver cirrhosis, decompensated liver disease, pregnancy or breast feeding, uncontrolled diabetes mellitus, significant retinal abnormalities, renal disease, BMI >30, ischemic heart disease, uncontrolled neuropsychiatric disease, autoimmune disease, organ transplantation, patients previously treated with interferon therapy. Seventeen patients were excluded from the study as they refused to be submitted to liver biopsy. Therefore only 483 patients (273 males and 210 females) were included in the study. At the time of inclusion in the study, all patients underwent:

History Taking and Clinical Examination: Full history was taken and through clinical examination was done.

Investigations included:

1. Laboratory: Hepatitis markers; testing for hepatitis B surface antigen (HBs Ag); using ARCHITECT HBs Ag qualitative reagent kit (1P97), Abbott Ireland and HCV antibody (HCV-Ab); using 6C37 ARCHITECT Anti-HCV reagent kit, Abbott Germany was done. Liver function tests; serum albumin, ALT, AST, total bilirubin, direct bilirubin, prothrombin time and concentration and serum glucose, creatinine, complete blood count (CBC), antinuclear antibodies, TSH, AFP, antibilharzial antibody were done. Polymerase chain reaction (PCR): Serum HCV RNA was extracted using an automated extraction system, HCV detection and quantification were performed using Abott Real-Time PCR assay (detection limit 50 IU ml).

2. Abdominal ultrasonography: The following data were recorded: Liver size: classified as shrunken (<11 cm), average (11-15 cm), or enlarged (>15 cm) Liver echogenicity: fine or coarse echopattern. Portal vein diameter and patency: The normal portal vein is up to 13 mm in diameter. Splenic size: normally, it is up to 12-13 cm according to its longest

axis. If the spleen was enlarged, it is classified as mild (13-16 cm), moderate (16-20 cm), or huge (>20 cm) splenomegaly *(Zwiebel, 2000, Olliff, 2003 and Bates, 2004)*.

3. Histological assessment: Four hundred and eighty-three liver biopsy samples were submitted to histological examination. In general, a sample of 1.5 cm in length that is 1.2-2 mm in diameter and contains at least 6-8 portal triads was considered adequate. Each was routinely-processed, formalin-fixed and paraffin-embedded. Five micron serial tissue sections were de-paraffinized, rehydrated and stained with hematoxylin and eosin (H&E) according to the usual schedule used in the laboratory to assess the degree of liver affection.

Necro-inflammatory activity (histological grade) and fibrosis (histological stage) were evaluated according to *Desmet et al. (1994)* with a pathologist who was blinded to the clinical conditions and laboratory results. Histological grade was performed taking into account the degree of necro-inflammation in the portal tracts, periportal and lobular areas: Grade 0 = no necro-inflammatory activity, Grade I = minimal activity, Grade II = mild activity, Grade III = moderate activity and Grade IV = severe activity. Stage of fibrosis was evaluated as: stage 0 = no fibrosis, stage I = portal fibrosis without septa, stage II = few septa, stage III = numerous septa delineating nodules without cirrhosis and stage IV = cirrhosis

Immunohistochemistery: For economical cause (the cost of the primary and secondary antibodies) only 50 specimens of formalin-fixed paraffin-embedded 5 micron tissue sections mounted on pre-cleaned (Superforest®*/Plus-fisherbrand® -USA) coated slides were immunostained using peroxidase-labelled streptavidine–biotin technique to detect the expression of CK7 and CK19. All the specimens were stained with each antibody.

Staining Procedure: Sections were deparaffinized in xylene and rehydrated through descending grades of alcohols, till distilled water. Endogenous peroxidase activity was blocked with 0.6% hydrogen peroxide (Cat # TP-015-HD, LAB VISION Corporation). The slides were washed in 20% diluted phosphate buffered saline; PBS (consists of 7.75 gm sodium chloride, 150 gm potassium phosphate diabasic anhydrous, 0.2 gm potassium phosphate monobasic anhydrous, and one liter of de-ionized water according to *Hussein et al. (2001)*.

Antigen retrieval was done to unmask the antigen sites by immersing the slides in unsealed plastic containers (Coplin staining jars CAT. NO.44208-000-USA) filled with sufficient amounts of antigen retrieval solution (10 mmol citric acid anhydrous buffer solution, pH 6.0 for CK7) or (1mmol ethylene diaminetetra-acetic acid; EDTA buffer solution pH 8.0 for CK19). The slides were microwaved at a high power for 5 minutes then at medium power for 15 minutes for CK19 and heat slides in hot air oven at 98ºC for 20 minutes for CK7, allowed to cool down, then washed in PBS.

Sections were incubated with 1/100 of 0.1ml mouse monoclonal antibodies for either CK7 (Ab-2, clone OV-TL 12/30, Cat# Ms-1352-P1ABX, LABVISION Corporation) or CK19 (Ab-1, cloneA53-B/A2.26, Cat# Ms-198-P1ABX, LAB VISION Corporation) in 1/100 normal goat serum; NGS 30 minutes at 40ºC for CK7 and 60 minutes at 40ºC for CK19. Excess reagent was thrown off and slides were rinsed in PBS. Tissue sections were treated with biotinylated goat serum (Cat # TP-015-HD, LAB VISION Corporation) for 10 minutes at room temperature and rinsed in PBS. Peroxidase-labelled streptavidin was applied for 10 minutes at room temperature and the slides were rinsed with PBS. Tissue sections were incubated with 14- diaminobenzidine (DAB) and 0.06 % H_2O_2 for 20 minutes, washed in distilled water and

counter-stained using Myers' Hematoxylin for 0.5 minute, washed in tap water, dehydrated in ascending grades of alcohol, cleared in xylol, left to dry, then mounted with DPX and cover slipped.

Positive and Negative Controls: Sections from ovarian carcinoma and from skin were used as positive controls for CK7 and CK19 respectively. Additional sections of the examined tissues were stained in parallel, but with omission of the primary antibody were used as negative controls.

Immunohistochemical Interpretation of CK7 and CK19: The number of HPCs was calculated by counting isolated CK7 and/or CK19 positive cells in the periportal area of the lobule. The mean of counted cells in three portal areas within the lobule was taken. Counted cells were smaller than normal hepatocytes *(Clouston et al. 2005)*.

4. Upper gastrointestinal endoscopic examination: If biopsy revealed stage III fibrosis, an upper gastrointestinal endoscopic examination was performed to exclude the presence of varices using Olympus, GIF-XQ 260 instrument.

5. Therapy: Patients included in this study were recruited to receive PEG-INF alfa-2a (180µg) and weight- based RBV therapy (1000 mg if <75 kg, 1200 mg if >75 kg) for 48 weeks.

6. Follow up of the patients: Adverse effects were monitored during each follow up visit; a. CBC and liver function tests every week during the first month then monthly till the end of the course, b. PCR at week 12 to assess the early virologic response (EVR) (decrease 2 log below the pretreatment level). Patients who will achieve EVR will continue till 24 weeks then another test will be done. Patients with negative viremia (detection limit 50 IU/ml) will continue till the end of the course. c. TSH level at 24th week and at the end of the course and at any time when hypothyroidism is suspected. At the end of the course patients were subjected to; a. PCR test and another one 6 months later to document SVR, b. Liver function tests and CBC.

ETVR was defined as undetectable HCV RNA (<50 IU/ml) at the end of treatment, whereas SVR was undetectable HCV RNA 6 months after the end of treatment. Patient relapse was defined as those whose HCV RNA was undetectable at the end of treatment but reappeared within 6 months of cessation of treatment. At 6 months after the end of treatment; response was evaluated and patients were categorized into two groups: patients with SVR and patients who didn't achieve SVR.

Statistical Analysis:

Data entry and analysis were done using SPSS software V11 (Chicago, USA). Continuous values were described by mean and standard deviation; SD. Categorical values were described by counts and proportions. Univariate analysis was performed using Student's t-test for continuous variables and Chi Square test; X2 test for categorical variables. This was followed by multivariate logistic regression analysis to identify the predictors of SVR. Correlations among the studied variables were tested by Spearman's correlation coefficient. Differences were considered statistically significant if P value was less than 0.05.

RESULTS

This study included 483 patients with chronic hepatitis C (273 males, 210 females) who fulfilled the study criteria were enrolled for this study. There were 53 (11%) patients who failed to complete the course due to side effects such as, neutropenia <250/mm^3, thrombocytopenia <25/mm^3 and psychosis, while 63 patients

(13%) failed to achieve EVR, 37 patients (7.7%) revealed stage 0 or stage IV hepatitis or had liver cirrhosis. So, only 330 patients (68.8%); 193 male and 137 completed the course and were included in the statistical analysis.

Subcutaneous injections of erythropoietin and granulocyte-colony stimulating factor; G-CSF were used in 115 patients (30% of patients who developed anemia) and 34 patients (10% of patients who developed leucopenia). Using drug dispensation sheets, compliance to treatment was calculated and all patients completed the course were adherent to therapy as they had taken at least 80% of the expected interferon and ribavirin dosage for 80% of the duration.

ETVR was achieved in 231 patients (70%) while 99 (30%) were non responders. Six months after the end of treatment patients were categorized into two groups according to their PCR status:

1. Patients who achieved SVR (n=132).
2. Those patients who didn't achieve SVR; non SVR including non-responders (n=99) and relapsers (n=99).

Demographic and laboratory characteristics of treated patients are summarized in Table (1). The patients were predominantly males (58%), with age range from 19-58 years and a mean age of 39.34± 15.6 years.

SVR was higher in female group and most of male patients didn't achieve SVR (P< 0.01). By univariate analysis, patients with SVR were significantly younger (P< 0.004) and had lower body weight (P<0.001). There was no relation between SVR and pretreatment viral load as assessed by PCR (P< 0.157). There was also no relation between SVR and pretreatment ALT level (P< 0.69). There was significant inverse relation between SVR and AST level (P< 0.000), and between SVR and AFP (P<0.000) as shown in Table (2). In a multivariate regression analysis, the independent predictors of SVR were younger age (P< 0.02) and lower serum AST (P < 0.02).

Table 1: Demographic and pre-treatment laboratory characterisics of 330 treated patients.

Variables	Minimum	Maximum	Mean ±SD
Age (ys)	19 years	58 years	39.34±15.61
Weight (Kg)	44	92	72.76±12.83
Body mass index (kg/m^2)	19	29	23±2.5
PCR (IU/ml)	100	84213567	3974697±16490522.27
AFP (ng/ml)	3	96	45.12±33.91
ALT (U/L)	4	100	42.64±30.26
AST (U/L)	2	97	44.38±31.37

Table 2: Relation between demographic, base line laboratory variables and SVR.

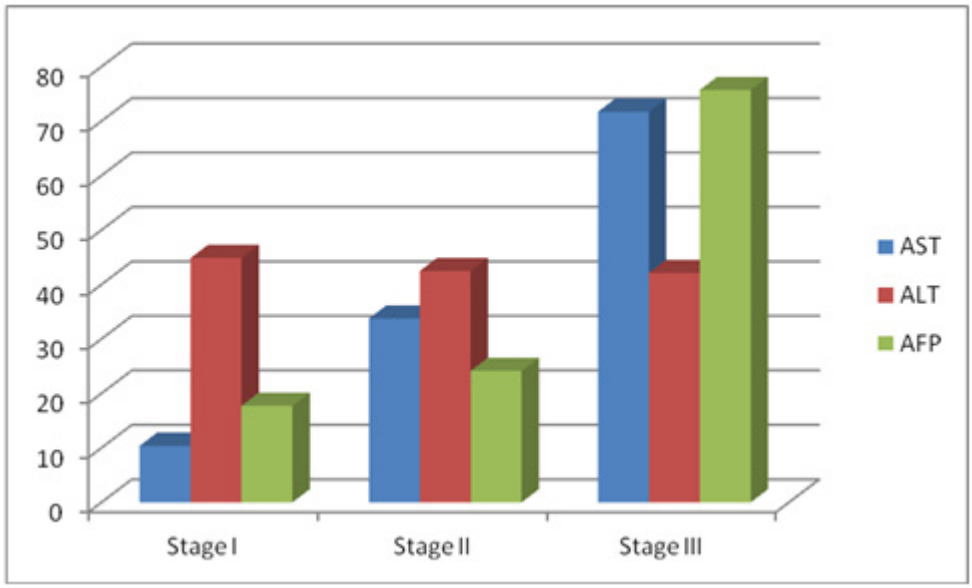
Variable	SVR		P value
	Yes	No	
Male	46 (35%)	147 (74%)	0.01*
Female	86 (65%)	51 (26%)	
<40 years	125 (95%)	8 (4%)	0.004*
>40 years	7 (5%)	190 (96%)	
Weight (Kg)	60.95±10.87	80.63±6.34	0.001*
Pre-treatment PCR (IU/ml)	608827±1001939.67	6218610.9±21113991.94	0.157
Pre-treatment ALT (U/L)	44.75±28.73	41.23±31.63	0.69
Pre-treatment AST (U/L)	15.2±8.95	63.833±25.13	0.000*
Pre-treatment AFP (ng/ml)	21.55±21.39	60.833±31.69	0.000*

Data are presented as n (%) or mean ± SD.

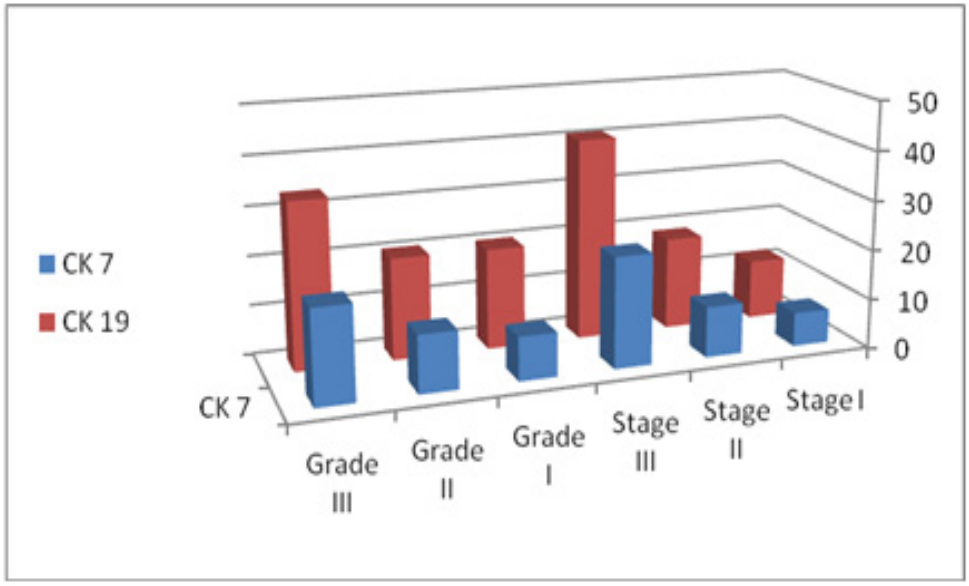
Histological assessment of the biopsy specimens were summarized in Table (3). There was significantly positive correlation between the grade of necro-inflammatory activity and the stage of hepatic fibrosis (P<0.003); with increasing grade of necro-inflammation, the stage of hepatic fibrosis became more prominent. There was no correlation between ALT and stage of hepatic fibrosis (P<0.58). There was significant positive correlation between AST and stage of hepatic fibrosis (P<0.000) and between AFP and stage of fibrosis (P<0.000) as shown in Graph (1).

Immunohistochemical stains for CK7 and CK19 showed cytoplasmic positivity in the HPCs and cells of bile ductules, while most of hepatocytes were negative. In those liver biopsy specimens, the average number of HPCs per portal tract ranged from 4 to 30 with a mean of 11±16.87 by CK7 and from 10 to 55 with a mean of 15.36 ±10.68 by CK19. Univariate statistical analysis showed significant association between increased HPCs as expressed by positive CK7 and CK19 on one hand and increased grade of necro-inflammatory activity on the other hand (P< 0.033, 0.026 respectively). There were significant associations between HPCs; CK7 and/or CK19 expressions on one hand and advanced stage of fibrosis on the other hand (P< 0.001, 0.000 respectively) as shown in Graph (2). Figure (1&2) showed CK7 and CK19 expressions decorating HPCs in different grades and stages of chronic hepatitis C infected patients.

No correlation was found between SVR and the grade of necro-inflammation (P<0.1), whereas, there was significant inverse relation between SVR and stage of hepatic fibrosis (P< 0.001). There was no relation between SVR and CK7 expression (P< 0.12), but there was high significant inverse relation between SVR and number of HPCs as assessed by CK19 expression (P< 0.000) as shown in Table (4).



Graph 1: Relation between ALT, AST and AFP and stages of chronic hepatitis.



Graph 2: Relation between markers of HPCs CK7 and/or CK19 and different grades and stages of chronic hepatitis.

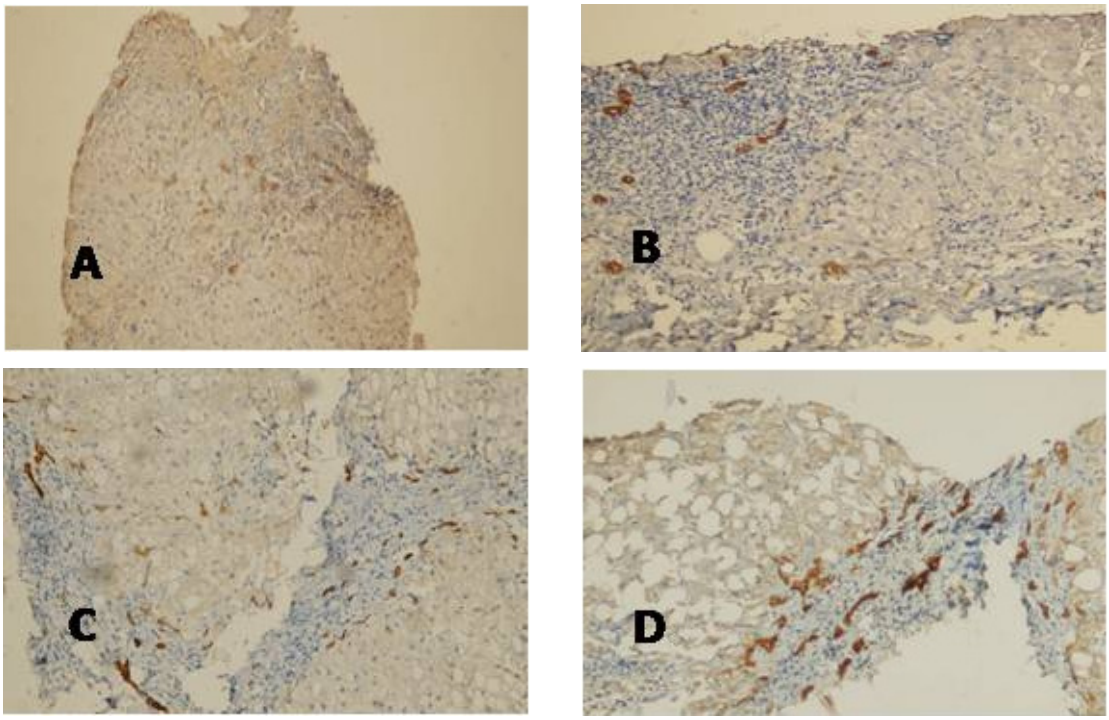


Figure 1: Mild, moderate and strong CK7 expression in HPCs and bile ductules in A; Grade I, Stage I X200 B; Grade II, Stage II X200 C; & D; Grade III, Stage III chronic hepatitis C infected patient. X200 A - C, X 400 D.

Table 3: Pre-treatment pathological characteristics of 330 treated patients.

Histological findings	Grade and stage	Number	Percentage
Grade	Grade I	46	14%
	Grade II	126	38%
	Grade III	158	48%
Stage	Stage I	86	26%
	Stage II	99	30%
	Stage III	145	44%

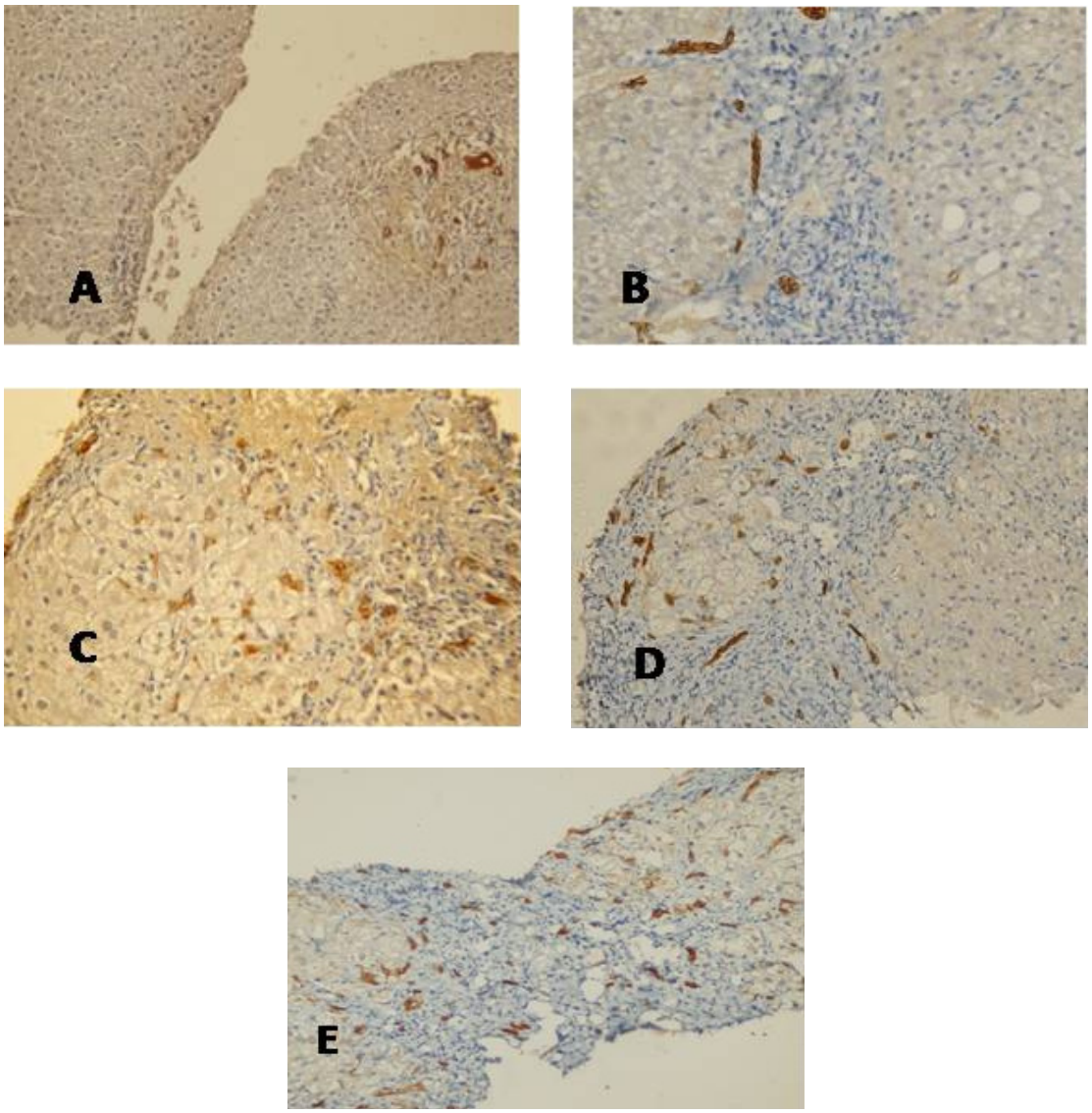


Figure 2: Mild, moderate and strong CK19 expression in HPCs and bile ductules in A; Grade I, Stage I X200 B; & C; Grade II, Stage II X200 D; & E; Grade III, Stage III chronic hepatitis C infected patient. X200 A & D, X 400 B, C & E.

Table 4: Relation between histological parameters and SVR in 50 patients.

Parameter	SVR (20)	non SVR (30)	P value
Grade			
Grade I	30 (23)	14 (7)	
Grade II	60 (45)	53 (27)	(NS)
Grade III	42 (32)	131 (66)	
Stage			
Stage I	11 (55)	2 (6.67)	
Stage II	7 (35)	8 (26.67)	0.001*
Stage III	2 (10)	20 (66.67)	
HPCs			
CK7 cells/ periportal area	11.7 ±15.29	16.7 ±11.17	(NS)
CK19 cells/ periportal area	14 ±11.78	35.36 ±20.68	0.000*

Data are expressed as n (%) or mean ± SD.

DISCUSSION

Treatment of chronic HCV has been considerably advanced since the initial identification of the virus in the late 1980s. Preliminary treatment with conventional interferon mono-therapy yield disappointing SVR of 10 to 15% *(Lauer and Walker, 2001)*. Since then, significant advances have been made in the treatment of HCV with reported SVR rates rising to greater than 50% with the use of PEG-INF alfa-2a and RBV combination therapy *(Abdoo and Lee, 2004)*.

Pretreatment predictors of response are useful for advising patients on their likelihood of SVR and absence of favorable factors should not be used to deny therapy. Some studies that compared patients with different doses of IFN-α have achieved different response rates *(Sarrazin, 2001 & Khokhar et al. 2002)*.

Our study showed that the SVR was achieved in 40 % of treated patients, this rate was low when compared with that (79.5%) reported by *Khokhar et al. (2002)* in Pakistan. The better response to treatment in the latter study may be due to the predominance of genotype 3a in Pakistan. On the contrary, *Sarrazin (2001)* reported lower SVR (31%). This decreased response to treatment may be due to the fact that genotype 1b was predominant in Germany. Derbala et al. *(2005)* found that the SVR in genotype 4 infected patients was lower among patients with high viral load.

Pegylated interferons have significantly improved pharmacokinetics resulting in improved antiviral efficacy, which also has the potential to alter the side effect profile. The most frequent side effects of treatment encountered in the current study were anemia (80%) followed by leucopenia (70%) then fatigue (60%) in agreement with *Manns et al. (2001)*. However, Al *Ashgar et al. (2008)* found lower frequencies of side effect; leucopenia (39%), anemia (36%) and fatigue in only (15%).

The relationship between the response to IFN-α treatment and the age is still controversial. In our study as well as in the study of *Garson et al. (1995)*, patients who had a complete response were significantly younger than those who had no response. In the current study multivariate analysis showed that an age > 40 years was one of the unfavorable predictors to IFN-α treatment response. On the contrary, *Bresci et al. (1993)* reported that older age was not an unfavorable

predictor for IFN-α treatment. Moreover, *Horiike et al. (1995)* reported that elderly patients with a low level of HCV RNA respond well to IFN-α treatment. They defined a response to IFN-α treatment as the return of aminotransferase levels to normal after the cessation of treatment irrespective of their HCV RNA status.

We noticed that most patients with advanced liver fibrosis were 40 years or older. The mechanisms behind the accelerated rate of progression with aging are poorly understood *(Di Martino et al. 2004)*. In agreement with *Hayashi et al. (1998)*, it has been shown that response to IFN-α treatment was better in women than in men. Interleukin (IL)-1β was significantly higher in patients with chronic HCV and then decreased with a complete response after the administration of IFN-α. This cytokine production may alter the effectiveness of IFN-α treatment *(Kishihara et al. 1996)*. IL-1β is stimulated by low concentrations of estrogen and progesterone *(Polan et al. 1988)*. Therefore, estrogen may be associated with sustained elimination of HCV in patients undergoing IFN-α treatment.

In the current study, a low body weight < 75 kg was associated with SVR which is in agreement with *Gheorghe et al. (2007)*. This may be attributed to diminished IFN-α bioavailability in obese patients because of decreased absorption of the drug when the subcutaneous route is used. Kroon et al. *(1991)* demonstrated that the skin fold thickness inversely correlates with the subcutaneous absorption of some drugs. Gheorghe et al. *(2007)* recommended that the negative impact of increasing weight on SVR can be overcome by treatment with PEG-IFN α-2b and RBV, if both drugs are dosed according to patient weight

Widjaja et al. *(2001)* and *Wenger et al. (2007)* found that the initial high body weight was an independent risk factor of treatment failure and weight reduction was associated with treatment success. On the contrary, *Al Ashgar et al. (2009)* didn't find correlation between SVR and weight of the patient.

In agreement with *Al Ashgar et al. (2009)*, we found that pre-treatment viral load was not a predictor of SVR. It is well known that viral load fluctuates and a single reading of HCV quantification may not reflect the actual viral load at the time of treatment and the differences in IFN response could be secondary to either a difference in the viral virulence and/or replication rate among different HCV genotypes and not the absolute viral load *(Hu et al. 2001)*. Hadziyannis et al. *(2004)* and *Xie et al. (2005)* found that the SVR rate was considerably higher in patients with a pre-treatment viral load of < 800.000 IU/ml.

Consistent with *Al Ashgar et al. (2009)*, we found no significant difference in SVR between patients with normal ALT and those with elevated ALT level. Stransky et al. *(2002)* found no relationship between liver histology and ALT level. Patients with normal ALT level may have histologically advanced liver disease and can respond to interferon therapy.

Consistent with *Al Ashgar et al. (2009)*, our study demonstrates that lower AST is an independent predictor of SVR to PEG-IFN α-2a and RBV. In a cohort of chronic HCV patients, *Assay* and *Minuk (2000)* reported a significant positive correlation between AST levels and the presence of advanced fibrosis. On the contrary, *Lu et al. (2003)* found that AST level was not related to fibrosis. Stransky et al. *(2002)* stated that fibrosis correlated more closely to serum activity of AST than serum activity of ALT in agreement with our findings. The relative increase in AST is probably related to both reduced clearance of AST by hepatic sinusoidal cells as well as to mitochondrial dysfunction *(Okuda et al. 2002)*.

We found that patients with SVR have significantly lower pretreatment serum AFP level

than that of the non responders in agreement with *Males et al. (2007)*; *Abdoul et al. (2008)*; *Al Ashgar et al. (2009)*. It was reported that the prevalence of increased serum AFP varies from10% to 43% in patients with chronic hepatitis *(Hu et al. 2001)*. Increased production of AFP in hepatitis was first thought to reflect the process of regeneration of surviving hepatocytes *(Males et al. 2007)*, but this hypothesis has been refuted before by *Goldstein et al. (1999)*.

Taketa *(1990)* reported that increased serum AFP level was due to hepatic damage associated with selective transcriptional activation of the AFP gene. In agreement with *Chu et al. (2004)* and *Chen et al. (2008)* we found significant correlation between elevated serum AFP and advanced stage of fibrosis. Tsamandas et al. *(2006)* found that HPCs were frequently present and express AFP protein/mRNA in liver biopsies taken from patients with HCV. This may provide another explanation of high serum level of AFP among non responders group who have increased HPCs expression and advanced fibrosis.

Hepatic fibrosis is a common response to chronic liver injury and might result in potentially lethal sequelae. At present, liver biopsy is the golden criterion for diagnosis of liver fibrosis *(Botta et al. 2003)*.

We found that patients with increased grade of necro-inflammation had higher stage of fibrosis in liver biopsy than did those with only mild grades of necro-inflammation in agreement with *Lu et al. (2003)*. On the contrary, *Niederau et al. (1998)* didn't confirm inflammatory activity; necro-inflammation as a risk factor for fibrosis.

In agreement with *Al Ashgar et al. (2009)* we found that there was no correlation between the grade of necro-inflammation and SVR. In contrast, *Derbala et al. (2006)* found positive correlation between higher grade of necro-inflammation and SVR.

Napoli et al. *(1996)* reported that the grade of inflammation correlates with the underlying immune response in the form of increased level of cytokines such as TNFα, INFγ and IL-2 responsible for hepatic inflammation. Therefore high grades of inflammation depicts higher immune response to HCV which responds more to immune modulation effect of IFN-α compared to low grade of inflammation, but these findings remains true for patient with cirrhosis only *(Hu et al. 2001)*. This may explain absence of relation between grade of necro-inflammation and treatment response in our study as patients with cirrhosis were excluded.

Coincide with results of *Myers et al. (2003)* and *Hasan et al. (2004)*, we found that the stage of fibrosis was significantly associated with SVR. Patients with low fibrosis stage were more likely to respond to therapy than those with higher stage. However, *Al Ashgar et al. (2009)* found no relation between stage of fibrosis and SVR. Certain cytokines may influence the inflammatory processes that are involved in both liver fibrogenesis and the antiviral response to IFN based therapy *(Nelson et al. 2000)*.

The activation of HPCs occurred in a variety of acute and chronic liver diseases *(Petersen et al. 1999)*, resulted in investigative efforts to precisely assess the nature, the site and cell type of origin of the HPCs that emerge in the liver after different types of injury *(Farber, 1956; Evarts et al. 1987; Thorgeirsson et al.1993; Fujio et al. 1994)*. Many investigators consider these oval cells as an immediate progeny of a putative hepatic stem cell *(Thorgeirsson et al. 1993)*.

We as well as *Tsmandas et al. (2006)* found HPCs in close association with fibrous tissue in the liver, often proliferating along the tracts from the expanded portal regions. HPCs were often observed in close association with inflammatory cells in patients with chronic HCV.

We assessed progenitor cell numbers on CK7, CK19-stained sections. In agreement with our results, *Tsmandas et al. (2006)* found that CK7 and CK19 were reliable markers for detecting HPCs. The numbers of CK19 HPCs were higher when compared with CK7 cells. Thus it seems that CK19 is a more sensitive marker for the recognition of HPCs and immunostaining for CK19 provides the best yield for such cells. On the contrary, *Libbrecht et al. (2000)* found that CK7 expression was better in detecting such progenitor cells.

Similar to *Libbrecht et al. (2000)* and *Fotiadu et al. (2004)*, our results showed that the progenitor cell number as assessed by CK7 and CK19, correlated significantly with the severity of necro-inflammation. The fact that the progenitor cell number increase in parallel to the degree of necro-inflammatory activity may suggest that cytokines produced by inflammatory cells such as TNF-α and IFN-γ act as growth factors promoting the proliferation of progenitor cells. This suggests a role for progenitor cells in hepatic regeneration *(Libbrecht et al. 2000)*.

Consistent with *Fotiadu et al. (2004)*, we reported that the CK7, CK 19 progenitor cell numbers correlated significantly with the stage of fibrosis. On the contrary, *Libbrecht et al. (2000)* didn't report any association between the progenitor cell number and stage of fibrosis.

The correlation of progenitor cell numbers with disease stage suggests their role in hepatic fibrogenesis *(Evarts et al. 1993 and Miyazaki et al. 1993)*. More recent studies using chemically injured rat liver show that activation of oval cells may coexist with activation of Kupffer and stellate cells *(Yin et al. 2002)*. It is becoming clear that progenitor cell/stellate cell interactions in hepatic diseases warrant further investigation.

Proliferating ductules detected by CK7 often occurs at the portal tract interface in patients with HCV and fibrosis *(Tan et al. 2002)*. These proliferating ductules are postulated to arise from HPCs *(Falkwoski et al. 2003)*. Roskams et al. *(2003)* hypothesized that ductular reaction contributes to periportal fibrogenesis.

To the best of our knowledge, the role of HPCs as a predictor of response to interferon therapy hasn't been studied before in the Egyptian patients. We found highly significant inverse correlation between SVR and number of HPCs as assessed by CK19 expression but no correlation between SVR and number of HPCs as assessed by CK7 expression. This suggests that CK19 provided the best yield of such cells.

As the progenitor cell numbers correlated significantly with stage of liver fibrosis and as fibrosis was inversely correlated with SVR in the current study this may explain the inverse correlation between SVR and number of HPCs.

The effect of IFN-α-based treatment in patients with chronic HCV was studied by Lim et al. (2006) who supposed that the reduced number of HPCs is mostly due to anti-proliferative effect of interferon, induction of apoptosis and promotion of differentiation.

CONCLUSIONS

Forty % of patients with chronic hepatitis C who completed the course of combination therapy achieved SVR. Younger age and lower pre-treatment AST are independent predictors of SVR. In chronic hepatitis C, progenitor cell activation is correlated with the grade and stage of disease. Proliferating HPCs as assessed by CK7 and CK19 expressions may play a role in hepatic regeneration occurring in this setting and could be incorporated in assessment of treatment response of those patients.

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الملخص العربي

مؤشرات الإستجابة الثابتة للعلاج بالانترفيرون في مرضي الإلتهاب الكبدي الفيروسي سي المزمن: دور الخلايا الكبدية الرائدة

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الخلفية: دراسة التنبؤ بمؤشرات الإستجابة الثابتة مع استخدام العلاج بالانترفيرون الفا المضاد للفيروسات طويل المدي وعقار الريبافيرين -٢ لعلاج المرضى الذين يعانون التهاب الكبد المزمن سي أمر حاسم لإختيار أولئك الذين يمكن أن يستفيدوا أكثر من غيرهم من العلاج. وقد وجد ان الزيادة في الخلايا الكبدية الرائدة في المرضى الذين يعانون التهاب الكبد المزمن سي له ارتباط بزيادة التليف والإستجابة للعلاج. ويمكن أن يتم الكشف عن الخلايا الكبدية الرائدة في الكبد عن طريق التعبير المناعى الهستوكيميائي للسيتوكيراتين ٧ والسيتوكيراتين ١٩.

وتهدف هذه الدراسة إلى: أ. تقييم معدل الإستجابة للعلاج على أساس الإنترفيرون مضاد الفيروسات في مرضى التهاب الكبد المزمن سي.
ب. الكشف عن التنبؤ بمؤشرات الإستجابة الثابتة للعلاج. ج. دراسة علاقات الإرتباط بين تعبيرالسيتوكيراتين ٧ والسيتوكيراتين ١٩ والإستجابة للعلاج.

المرضى والأساليب: وقد شملت هذه الدراسة ٤٨٣ مريضا بالتهاب الكبد المزمن سي الذين إستوفوا معايير الدراسة وقد خضعوا للتقييمات السريرية، والبيوكيميائية والفيروسية قبل العلاج وبعد ١٢ و ٢٤ و ٤٨ و ٧٢ أسبوعا بعد العلاج. وقد إستوفى العلاج ٣٣٠ مريضا فقط حيث أدرجوا في التحليل الإحصائي. تم فحص تعبير السيتوكيراتين ٧ والسيتوكيراتين ١٩ باستخدام تقنية البيروكسيديز أفيدين، بيوتين المناعى، في عينات ٥٠ مريضا فقط.

النتائج: وتم تحقيق مؤشرات الإستجابة الثابتة للعلاج في ٣٣٠/١٣٢ (٤٠%)) من المرضى. وكان ذلك أعلى احصائيا في المرضى الإناث، وفي الفئة العمرية الأصغر سنا، والمرضى الذين كان وزن أجسامهم أقل. وكانت هناك علاقة ذات دلالة إحصائية عكسية بين مؤشرات الإستجابة الثابتة للعلاج ومستوى كل من إنزيم الاسبرتات امينوترانسفيريز والألفا فيتوبروتين. وكانت التنبؤات المستقلة عن مؤشرات الإستجابة الثابتة للعلاج هي كل من السن الأصغر والمستوى الأقل لإنزيم الاسبرتات امينوترانسفيريز. وكان هناك إرتباطا مهما بين تعبيرات السيتوكيراتين ٧ و/أو السيتوكيراتين ١٩ من ناحية ودرجة الإلتهاب النخري من ناحية اخرى، وكذلك و/أو المراحل المتقدمة من التليف. وكانت هناك علاقات عكسية كبيرة بين مؤشرات الإستجابة الثابتة للعلاج من ناحية ومرحلة التليف الكبدي، وتعبيرالسيتوكيراتين ١٩ من ناحية أخرى.

الإستنتاجات: وقد تحققت مؤشرات الإستجابة الثابتة للعلاج في أربعين في المائة من المرضى الذين يعانون من الإلتهاب الكبدي المزمن سي الذين أكملوا دورة العلاج المركب. وقد كان السن الأصغر والمستوى الأقل لإنزيم الاسبرتات امينوترانسفيريز قبل المعالجة هي نبؤات مستقلة عن مؤشرات الإستجابة الثابتة للعلاج. وفي الإلتهاب الكبدي المزمن سي يرتبط تنشيط الخلايا الرائدة مع درجة الإلتهاب ومرحلة التليف في المرض. وقد يلعب تكاثر الخلايا الكبدية الرائدة وفقا لتقييم تعبيرات السيتوكيراتين ٧ و/أو السيتوكيراتين ١٩ دورا في تجديد الكبد في هذا السياق، ويمكن إدراجها في تقييم الإستجابة للعلاج في هؤلاء المرضى.