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RESEARCH ARTICLE

Sex Hormones, Prolactin, and Platelet Indices as Predictors of Sustained Virological Response in Male Patients with Chronic Hepatitis C Genotype 4 Receiving Combined Antiviral Therapy (Pegylated Interferon and Ribavirin)

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Abstract

Background: Hepatitis C virus (HCV) is one of the most noxious infectious diseases; however the impact of gender on the response to HCV still poorly understood. **Aim:** We aimed to investigate platelet indices, testosterone and prolactin levels as predictors of sustained virological response (SVR) in chronic hepatitis C (CHC). ***Methods:*** Liver biopsy, serum testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin and platelet parameters were evaluated to 86 men with CHC who were divided into 51 patients with early fibrosis and 35 patients with late fibrosis. Patients receiving pegylated interferon and ribavirin for 48 weeks; then follow up for 6 months. ***Results:*** Patients with early fibrosis showed lower total testosterone and higher free testosterone levels (P<0.001) than those with higher fibrosis scores. A univariate analysis showed that age, viral load, liver fibrosis, platelet count and indices, total and free testosterone and prolactin levels were associated with SVR. In a multivariate analysis, liver fibrosis (F0–2), platelet count ≥140 x10^3/mm^3, mean platelet volume (MPV) ≤ 8.5 fL, total testosterone ≤ 17.5 nmol/L, free testosterone ≥ 0. 07 nmol/L and prolactin ≤ 185 mIU/L were independently associated with SVR. ***Conclusion:*** Higher levels of platelet count and free testosterone levels as well as lower levels of total testosterone, MPV and prolactin levels may be good predictors of SVR. We recommend screening for thrombocytopenia; total testosterone deficiency and prolactin elevation in CHC infection should selectively target men with more severe liver disease or documented higher fibrosis stages.

Introduction:

It is estimated that 3% of the world population chronically infected with hepatitis C virus (HCV). Infection by this virus is responsible for 20% of acute hepatitis, and 70% of chronic hepatitis (Negro and Alberti, 2011). Chronic hepatitis C is one of the main causes of hepatic cirrhosis, and hepatocellular carcinoma (HCC) (Davis et al. 2003). Prevalence of antibodies against HCV (anti-HCV) is variable. Developing countries enclose between 1% and 2% of this population. A higher prevalence has been reported for Europe and Africa, and it is estimated that 15% of the Egyptian population is seropositive (EI-Sayed et al., 1996) and high seroprevalence of hepatitis C infection among risk groups in Egyptian people (Abdel-Wahab et al., 1994).

Males have strikingly increased risk of advanced liver disease (e.g., cirrhosis and HCC) across varied disease etiologies, including HCV, thus supporting the potential roles for gender-associated differences in risk factor exposures as well as sex-based biological differences in disease progression (Yu et al., 2001).

Previous studies proved that total testosterone levels were reduced in patients with decompensated cirrhosis and hepatocellular carcinoma. Specific mechanisms implicated are suppression of gonadotrophin release and increased peripheral aromatization of testosterone to oestradiol (Pignata et al., 1997). However, the effect of compensated liver disease on sex hormone levels and the relationship between liver architectural damage and sex hormone levels is not well characterized.

Recent study demonstrates that chronic hepatitis C is associated with a variable degree of thrombocytopenia. As the disease advances, the platelet count decreases and, in most cases, both mechanisms are involved. The stage of fibrosis is one of the major determinants of thrombocytopenia (Olariu et al., 2010). In addition to their function in haemostasis, platelets play an important role in the inflammatory response (Mirsaeidi et al., 2010). Changes in platelet counts during infections are reported to be associated with enhanced disease severity and mortality (Boos et al., 2007). Furthermore, platelet activation alters the morphology of these cells, which can be evaluated on the basis of mean platelet volume (MPV) and platelet distribution width (PDW) (Jackson, Carter, 1993).

Moreover; the effect of Interferon alfa (IFN-α) as a treatment for hepatitis C on thyroid function, with thyroid under- or over activity, have been described (Marcellin et al., 1992). However; the effect of IFN-α on sex hormones is less well understood. In clinical studies, Orava et al.(1986) showed a transient reduction of plasma total testosterone in men during IFN-α treatment.

The aim of our study was to investigate platelet indices (MPV & PDW), testosterone and prolactin levels as predictors of sustained virological response (SVR) in the context of hepatitis C infection.

Patients and methods:

A cohort study was done on consecutive 101 male patients referred to outpatient clinic of Tropical Medicine Department (Mansoura University-Egypt) with hepatitis irrespective to their etiologies between April, 2012 and June, 2014. Only 86 men with CHC were selected to be enrolled in our study and they were divided according to the results of liver biopsy into two groups; group I with early fibrosis (fibrosis score ≤ 2) (n=51& age: 43.9 ± 10.3 years old) and group II with late fibrosis (scores >2) (n=35& age: 46.3 ± 8.3 years old).

Exclusion criteria included HCV infected patients who were negative for genotype IV, patients with history of co-infection with either human immunodeficiency virus (HIV) or HBV, liver transplant, decompensated liver disease, HCC, psychosis or dementia, patient with testicular cancer or under treatment of cancer prostate, patients receiving estrogen or androgen therapy or other gonadal hormone agonist/antagonists. Exclusion criteria also included patients with alcohol intake; immunosuppressant drugs, patients with fasting blood glucose level > 6.2 mmol/L or under anti-diabetics, patients who undergoing dialysis, with clinically overt hypo- or hyperthyroidism. Moreover; patients with heart failure, hypertension, hyperlipidemia, peripheral vascular disease or autoimmune diseases were also excluded from this study. A total of 15 patients were excluded from this study (2 patients with CHB, 3 patients with combined HCV and HBV, 2 patients with autoimmune hepatitis, 3 patients with probable drug-induced hepatitis**,** 2 patients with nonalcoholic steatohepatitis (NASH), one patient with uncontrolled hyperthyroidism, one case with SLE and one patient with renal failure on dialysis).

All studied patients were submitted to complete history taking; clinical examination, radiological and laboratory assessment, and ultrasound guided liver biopsy.

Sampling:

- After 8-hour of overnight fast; 10 ml venous blood samples were obtained from all studied patients (1ml on EDTA for complete blood picture (CBC), 1.8 ml on sodium citrate for INR, 2ml in sterile tube for HCV-PCR and 5 ml without anticoagulants for serum samples).
- CBC and coagulation profile were analyzed immediately while serum samples were divided into aliquots and frozen at -20°C till analysis.

Methodology

All the following were done to all patients:

- **Anthropometric measurements:**

Anthropometric measurements, including the height and weight for calculating the body mass index (BMI), were recorded at baseline and at 6 months during therapy and 6 months after finishing combined antiviral treatment and the BMI was calculated as weight (in kilograms) divided by height (in square meters). Overweight and obesity was defined as a BMI in the range of 25 to 30 kg/m2, and ≥30 kg/m2 respectively (Ogden et al., 2006).

- **Laboratory assessment included:**

Liver function tests including (AST, ALT, total bilirubin, serum albumin and alkaline phosphatase), serum creatinine and blood glucose (fasting and postprandial); all were measured on a Dimension Xpand plus chemistry analyzer using its kits both were supplied by Siemens Technology (USA), prothrombin time and INR were determined using kits supplied by Siemens Technology (USA), complete blood count was measured on CELL-DYN Emerald cell counter (ABBOTT, Wiesbaden, Germany), total testosterone and free testosterone were measured by enzyme-linked immunosorbent assay (ELISA) kits supplied by BioVendor R&D (England), serum prolactin and LH were assayed by ELISA kits supplied by Cayman chemical company (USA), FSH was measured by ELISA kit supplied by Alpaco (USA).

The expected reference ranges (according to measuring kits) for men aged 20–55 years are 10.4- 41.6 nmol/L for total testestrone, 0.02-0.12 nmol/L for free testosterone, 38-360 mIU/L for prolactin, 0.7–7.4 IU/L for LH and 1-18 IU/L for FSH. the expected normal ranges of MPV were 6.5–11.5 fl., PDW, 10–18 fl.

As for treatment, subcutaneous PEG IFN-α (1.5 µg/ kg/week) and oral ribavirin (800- 1200 mg/day) were administered to genotype 4 patients for 48 weeks. The hormone levels and liver function tests were determined at base line (before therapy), at 6 months during therapy and 6 months after finishing combined antiviral treatment.

During monitoring of patients, the dose of ribavirin was decreased when hemoglobin values fell below 10 g/dL, and the agent was discontinued in cases with hemoglobin levels below 8.5 g/dL. Pegylated interferon was decreased to half of the initial dose when the neutrophil count was below 750 cells/mm^3 and the platelet count <50,000 cells/mm^3. A decrease of the neutrophil count below 500 cells/mm^3 and the platelet count below 25,000 cells/mm^3 led to discontinuation of treatment. Patients who achieved SVR were followed up. Recurrence of disease during the follow-up period was regarded as relapse (No SVR). Patients with severe adverse events associated with treatment and patients who failed to achieve ETR were regarded as treatment failures. Patients who voluntarily stopped treatment or who were lost to follow up were not included in the evaluations.

- **Assessment of HCV RNA viral load:**

HCV viral load in the plasma was measured in all patients at baseline and then at weeks 12, 24, 48, and 72 by a real time RT-PCR. Qiagen Viral RNA Kit (Hilden, Germany) was used for HCV RNA extraction and HCV RT-PCR TaqMan reagent (Applied Biosystem, USA) on ABI Prism 7000 Sequence Detection System (Applied Biosystem, USA) was used for real-time PCR detection. (Detection limit of the PCR is 12 IU/ml).

- **Detection of HCV genotype IV:**

Extraction of HCV RNA from patient's sera was done by Qiagen Viral RNA mini Kit (Hilden, Germany). The core region was first amplified by RT-PCR in the thermocycler (Biometra Analytik Jena Company, Germany) using specific primers common to all genotypes (Ohno et al., 1997). The product was then amplified in the second PCR with type IV genotype specific primers (Ohno et al., 1997) and Taq DNA polymerase (Qiagen, Hilden, Germany). HCV genotype IV was determined by comparing the amplified product with 100 bp DNA ladder marker (Life Technologies, USA). Genotype IV-specific band was detected at position 99 bp.

- **Histopathology of percutaneous ultrasound guided liver biopsy:**

Liver biopsies were analyzed after paraffin embedding, 5 µm sections were obtained for hematoxylin and eosin staining, Prussian Blue staining, and Masson's trichrome staining. Each liver tissue sample was diagnosed on the basis of the presence of at least 10 complete portal tracts has long been considered the 'gold standard' to determine liver histology, disease activity and liver fibrosis (Colloredo et al., 2003). In the present study, the degree of histologic hepatic fibrosis and inflammation was scored using the METAVIR scoring system (Bedossa et al., 1996). Based on the degree of lymphocyte infiltration and hepatocyte necrosis, the level of inflammation was classified from A0 to A3, with a higher score indicating more severe inflammation. Fibrosis was graded from F0 to F4 as follows: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Steatosis was quantified as the percentage of hepatocytes that contained fat droplets classified into three groups: <5%, 5-30% and >30% (Brunt et al. 1999). Liver biopsy was assessed by a pathologist (blinded to clinical and laboratory data).

All demographic and laboratory data were collected at the time of the liver biopsy.

Efficacy, assessment, and end points of standard combination therapy:

All patients of both groups received combined therapy (pegylated IFN-alfa and ribavirin therapy). HCV RNA viral load was assessed at week 12 [early virological response (EVR)], at week 24, and at week 48 of treatment [end-of-treatment response (ETR)]. Efficacy of therapy was assessed with sustained virological response (SVR), which was defined as the undetectable HCV RNA by real-time PCR at week 24 of the post-treatment follow-up. Patients were considered non responders (NR) and therapy was discontinued if HCV RNA level decreased Less than 2 log10 IU/ml from baseline at week 12 of therapy and if any HCV RNA was still detectable at week 24. Patients with an ETR who were seroreverted to HCV RNA during follow-up were classified as relapsers (No SVR) [Petta et al., 2009]. In addition; patients with virological breakthrough were excluded also from the study. According to these rules; only 64 of treated patients became responders (ETR) and they were included in the results in table 2; as follow up for 6 months after finishing treatment enable us to divide patients as SVR group and non-SVR group; while 22 of them were excluded; as follow: 12 patients were non responders (NR), in addition to 5 patients showed virological breakthrough, 5 patients showed no drug adherence due to severe side effects (2 patients with severe neutropenia, 2 patients with severe recurrent anemia and 1 patient with sever thrombocytopenia).

Safety Assessments:

Safety was assessed at scheduled clinic visits by physical examinations, laboratory tests, and reports of clinical adverse events; then treated according to schedule of the study.

Ethics:

This study was approved by the Ethical Committee of Mansoura University and all patients provided written informed consent prior to participation in any protocol-specific procedures. The study was conducted in accordance with the guidelines of the Helsinki Declaration.

Statistical Analysis:

All statistical analyses were performed using the SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA). Data were first tested by Kolmogorov–Smirnov test for distribution of data. Parametric data was expressed in mean and standard deviation (SD). The mean and SD of the differences and the limits of agreement, defined as the mean ± 2 SD of the difference (95%CI), were calculated. Unpaired t test was used for intergroup comparisons. A P-value of less than 0.05 indicated statistical significance. Correlations between numerical data were determined with the Pearson's rank correlation coefficient. Variables with $P < 0.05$ at univariate analysis were retained for the multivariate logistic regression analysis. Stepwise and multivariate logistic regression models were used to explore the independent factors that could be used to predict a virological response. The significance of trends in values was determined with the Mantel–Haenszel chi-square test.

Results:

In our study; we found significant increase in platelet count of men with early fibrosis score than men with late fibrosis score ($172 \pm 85(x10^3/mm^3)$ vs. $135 \pm 64(x10^3/mm^3)$ and $P=0.032$, while there was significant increase in MPV and ALT in men with late fibrosis score than men with early fibrosis score ($P= 0.011$ and $P <0.001$

respectively). No statistical significant difference in age, BMI, plasma HCV RNA viral load, liver activity score, PDW, AST, Albumin, haemoglobin and TLC (total leukocyte count) between two groups (all $P<0.05$) (Table 1).

Patients with early fibrosis showed lower total testosterone (16.1 ± 5.9 vs. 23.7 ± 7.8 nmol/L, P<0.001) and higher free testosterone levels (0.046± 0.01 vs. 0.032 ± 0.009 nmol/L, P <0.001) than those with higher fibrosis scores. But, there were no significant difference in serum levels of prolactin, FSH and LH between two groups (all $P<0.05$) as shown in table 1.

Biochemical and hormonal changes in patients who achieved ETR: are summarized in table 2, figure 1 and figure 2:

The evaluation of treatment outcomes revealed the following: ETR was achieved in 74.4% (n=64) of patients, 25.6% (n=22) of patients were evaluated as unresponsive.

In group 1, 39 patients achieved ETR show platelet count was 173±74 ($x10^3/mm^3$), 142±93 ($x10^3/mm^3$) and 155±55 ($x10^3/mm^3$), respectively ($P = 0.001$) for the 'at base line-at 6 months treatment' and ($P = 0.045$) for the 'at base line -final' comparisons). MPV was 7.8±1.4 fL, 6.4±1.3 fL and 7±1.4 fL, respectively ($P = 0.001$) for the 'at base line-at 6 months treatment' and ($P = 0.045$) for the 'at base line -final' comparisons). Serum albumin levels were within the normal range throughout the study period. There was a transient reduction in total testosterone from 20.1 ± 7.3nmol/L for patients at base line therapy to 14.6 ± 2.4 nmol/L at 6 months of treatment ($P = 0.003$), which recovered to 19.1 ± 3.7 nmol/L post-therapy; also there was a transient reduction in free testosterone from 0.083±0.009 nmol/L for patients at base line therapy to 0.055 ± 0.004 nmol/L at 6 months of treatment ($P<0.001$), which recovered to 0.076 ± 0.012 nmol/L post-therapy.

During the study period; serum prolactin concentrations rose significantly from 104±18 mIU/L for patients at base line therapy to 135±25 mIU/L at 6 months of treatment (P<0.001), which recovered to 120±17 mIU/L post-therapy (p=0.005). However; serum FSH and LH did not change significantly during IFN treatment.

In group 2, 25 patients achieved ETR show platelet count was 153±89 ($x10^3/mm^3$), 131±72 ($x10^3/mm^3$) and 148±65 ($x10^3/mm^3$), respectively ($P = 0.001$) for the 'at base line-at 6 months treatment' and ($P = 0.065$) for the 'at base line -final' comparisons). MPV was 8.5±1.4 fL, 7.2±1.1 fL and 8.2±1.3 fL, respectively ($P = 0.001$) for the 'at base line-at 6 months treatment' and ($P = 0.43$) for the 'at base line -final' comparisons). Serum albumin levels were within the normal range throughout the study period. There was a transient reduction in total testosterone from 25.1 ± 8.4 nmol/L for patients at base line therapy to 16.7 ± 2.5 nmol/L at 6 months of treatment ($P< 0.001$), which recovered to 23.2 ± 4.3 nmol/L post-therapy; also there was a transient reduction in free testosterone from 0.058±0.008 nmol/L for patients at base line therapy to 0.029 ± 0.002 nmol/L at 6 months of treatment ($P< 0.001$), which recovered to 0.044 ± 0.003 nmol/L post-therapy ($P< 0.001$).

During the study period; prolactin blood concentrations rose significantly from 115±25 mIU/L for patients at base line therapy to 190±33 mIU/L at 6 months of treatment ($P< 0.001$), which recovered to 130±22 mIU/L post-therapy. However; serum FSH and LH did not change significantly during IFN treatment.

Predictors of sustained virological response:

Both pretreatment and treatment factors that could be associated with the response to Peg-IFN and ribavirin combination therapy were compared between patients with and without SVR in Table 3. This univariate analysis showed that age ($P = 0.008$), baseline viral load level ($P= 0.018$), fibrosis stages (0-2) ($P=0.015$), platelets counts ($P = 0.002$), MPV, serum total testosterone, serum free testosterone and serum prolactin (all P <0.001) contributed to achievement of SVR. Factors that were significantly associated with SVR by univariate analysis were then analyzed by multivariate logistic regression analysis. The multiple logistic regression analysis showed that liver fibrosis (F0–2) [odds ratio (OR): 1.67, 95% confidence interval (CI): 0.54—5.83, p=0.038], Platelet Count ≥140 $x10^3/mm^3$ [(OR) 11.44, 95% (CI) 1.050-1.125, P = 0.035], MPV ≤ 8.5 fL [(OR) 0.875, 95% (CI) 0.221–0.681, P = 0.01], total testosterone ≤ 17.5 nmol/L [(OR) 0.401, 95% (CI) 0.221–0.681, P = 0.01], free testosterone ≥ 0.07 nmol/L [(OR) 1.007, 95% (CI) 1.061–1.853, P = 0.01] and prolactin ≤ 185 mIU/L [(OR) 0.972, 95% (CI) 0.952-0.974, P = 0.01] were independently associated with SVR (Table 4).

Table 1 Demographic data and laboratory findings of studied patients (n=86):

characteristic	Group I (n = 51)	Group II (n = 35)	P value
Age (years)	43.9 ± 10.3	46.3 ± 8.3	0.255
BMI (kg/m2)	21.9 ± 3.2	22.2 ± 3.1	0.67
Plasma HCV RNA load ($x10^5$ IU/ml)	5.95 ±2.7	6.32 ±2.1	0.49
Liver Activity grading (A 0–1/2–3)	31/20	21/14	0.75
Platelets ($x10^3/mm^3$)	172 ± 85	135 ± 64	0.032
Platelet Indices:			
MPV (fL)	7.5 ± 1.5	8.6 ± 2.4	0.011
PDW (fL)	15.5 ± 1.7	16.1 ±0.9	0.059
ALT (IU/L)	45 ± 33	78 ± 23	<0.001
AST (IU/L)	59±31	66±22	0.253
Albumin (g/dl)	4 ±1.1	3.9±1.2	0.69
Haemoglobin (g/dL)	12.5 ± 2.2	11.9 ± 3.3	0.314
TLC ($/mm^3$)	5035 ± 1135	4720 ± 1420	0.257
Hormonal assay:			
Total testosterone (nmol/L)	16.1 ± 5.9	23.7 ± 7.8	<0.001
Free testosterone (nmol/L)	0.046± 0.01	0.032 ± 0.009	<0.001
Prolactin (mIU/L)	195 ± 88	210±54	0.372
FSH (IU/L)	8.9±2.1	9.8±2.5	0.075
LH (IU/L)	3.9±0.5	4.1±0.45	0.061

Group I: Men with early fibrosis score
Group II: Men with late fibrosis score

BMI, Body mass index; MPV, Mean platelet volume; PDW, Platelet distribution width; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TLC: Total Leukocyte Count; FSH, Follicle-stimulating hormone ; LH, Luteinizing hormone

Table 2 Effect of standard combined therapy on biochemical and hormonal levels in patients who achieved ETR (n=64):

	characteristic	At base line (before therapy)	At 6 month of therapy	Post-treatment (6 months)
Group 1	ALT (U/L)	55±22.5	34.5±14.6**	36.5±11.8*
	Platelets ($x10^3/mm^3$)	173±74	142±93**	155±55*
	MPV (fL)	7.8±1.4	6.4±1.3**	7±1.4*
	Albumin (g/dl)	4.1±0.8	3.9±1.2	3.8±0.9
	Total testosterone (nmol/L)	20.1 ± 7.3	14.6 ± 2.4*	19.1 ± 3.7
	Free testosterone (nmol/L)	0.083±0.009	0.055 ± 0.004**	0.076 ± 0.012**
	Prolactin (mIU/L)	104±18	135±25**	120±17*
	FSH (IU/L)	4.2±1.3	4±1.1	3.9±0.8
	LH (IU/L)	3±0.3	2.9±0.3	3.1±0.2
Group 2	ALT (U/L)	96±22.5	58.5±20.8**	44.5±19.5**
	Platelets ($x10^3/mm^3$)	153±89	131±72**	148±65
	MPV (fL)	8.5±1.4	7.2±1.1**	8.2±1.3
	Albumin (g/dl)	3.9±0.8	3.7±1.1	3.8±0.7
	Total testosterone (nmol/L)	25.1 ± 8.4	16.7 ± 2.5**	23.2 ± 4.3**
	Free testosterone (nmol/L)	0.058±0.008	0.029 ± 0.002**	0.044 ± 0.003**
	Prolactin (mIU/L)	115±25	190±33**	130±22
	FSH (IU/L)	5.5±1.1	4.8±1.3	5.1±0.9
	LH (IU/L)	3.3±0.4	3.1±0.2	3.2±0.3

Paired t-tests comparing at 6 month of therapy and Post-treatment to baseline values with a significant two-tailed $P < 0.05$

*P value < 0.05

**P value < 0.001

ALT, alanine aminotransferase; MPV, Mean platelet volume; FSH, Follicle-stimulating hormone ; LH, Luteinizing hormone

Table 3 Factors associated with SVR among studied patients– univariate analysis:

Factors	SVR (n = 48)	No-SVR (n = 38)	*P value*
Age (years)	44.9 ± 10.3	50.3 ± 7.3	0.008
BMI (kg/m2)	22.9 ± 3.2	22.6 ± 3.1	0.66
Plasma HCV-RNA load ($x10^5$ IU/ml)	5.53 ±2.2	6.81 ±2.7	0.018
Fibrosis (F 0–2/3–4)	42/6	12/4	0.015
Activity (A 0–1/2–3)	31/17	11/5	0.75
Platelet count ($x10^3/mm^3$)	192± 60	150 ± 62	0.002
Platelet Indices:			
MPV	7.2 ± 0.55	8.5 ± 0.44	<0.001
PDW	15.7 ± 1.7	16.1 ±0.9	0.193
ALT (IU/L)	69 ± 31	75 ± 33	0.389
Haemoglobin (g/dL)	12.6 ± 1.2	12.9 ± 1.3	0.27
TLC ($/mm^3$)	5155 ± 1575	4830 ± 1650	0.355
Hormonal assay:			
Total testosterone (nmol/L)	16.1 ± 4.8	22.6 ± 5.6	<0.001
Free testosterone (nmol/L)	0.076± 0.01	0.041 ± 0.009	<0.001
Prolactin (mIU/L)	166 ± 75	255±41	<0.001
FSH (IU/L)	9.2±2.4	9.7±2.8	0.375
LH (IU/L)	3.88±0.7	4.1±0.5	0.106

BMI, Body mass index; MVP, mean platelet volume; PDW, Platelet distribution width; ALT, alanine aminotransferase; TLC: Total Leukocyte Count; FSH, Follicle-stimulating hormone ; LH, Luteinizing hormone

Table 4 Factors associated with SVR among patients who completed the treatment – multivariate analysis:

Variables	Odds ratio	95% CI	*P*-value
Age (years) ≤ 40 years	1.571	0.31—4.61	0.14
Fibrosis (F 0–2)	1.67	0.54—5.83	0.038
Plasma HCV RNA load ≤ ($5x10^5$ IU/ml)	1.64	0.62—2.30	0.22
Platelet Count ≥140 $x10^3/mm^3$	11.44	10.50-11.25	0.035
MPV ≤ 8.5 fL	0.875	0.221–0.681	0.01
Total Testosterone ≤ 17.5 nmol/L	0.401	0.221–0.681	0.01
Free Testosterone ≥ 0. 07 nmol/L	1.007	1.061–1.853	0.01
Prolactin ≤ 185 mIU/L	0.972	0.952-0.974	0.01

MVP, mean platelet volume; CI, confidence interval

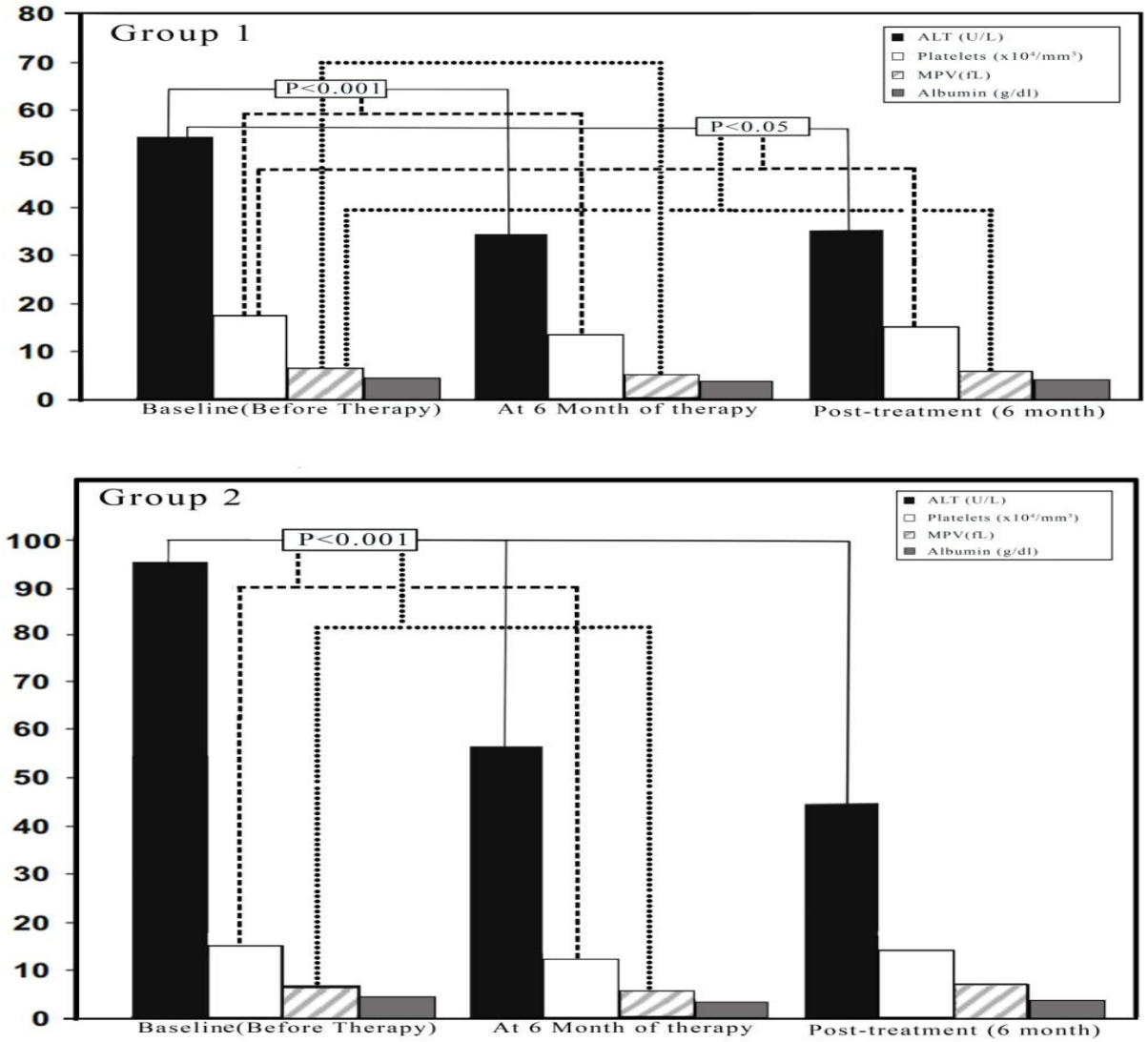

Figure 1 Biochemical changes (ALT, platelet count, MPV and serum albumin) at baseline, at 6 month of therapy and post-treatment (6 months) in studied groups.

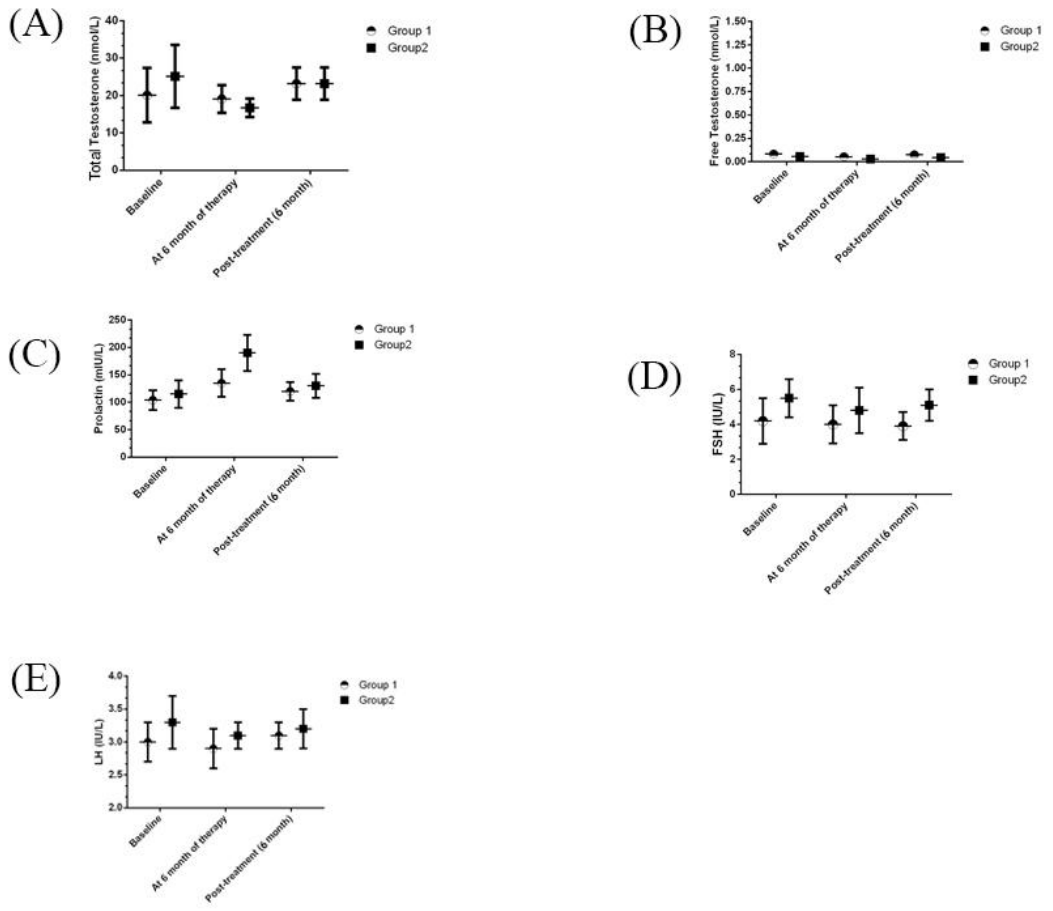

Figure 2 Total testosterone (A), free testosterone (B), prolactin (C), FSH (D) and LH (E) changes at baseline, at 6 month of therapy and post-treatment (6 months) in studied groups.

Discussion:

Egypt represents the world's highest prevalence of HCV infections (Elkady et al., 2009). Besides, genotype-4a of the virus, which is the common strain in Egypt, responds poorly to the pegylated interferon alpha plus ribavirin (PEG-IFN-α/RBV) combination therapy (Simmonds et al., 2005). Moreover, dual therapy with PEG-IFN-α /RBV has markedly improved the clinical outcome, but about half of the infected patients expected to respond favorably to currently available treatment (Watanabe et al., 2007). Staggeringly, some hormones play a significant role in the immune cells regulation such as; B-cells, T-cells, and natural killer cells (Liu et al., 1997), this role gives them the possibility of the chronic liver disease resistance and attack from the early stages of infections (Picardi et al., 2003). Recently, authors (Plockinger et al., 2007) have examined the impact of HCV infection and IFN-based therapy on the growth hormone (GH), prolactin and testosterone concentrations among HCV infected patients.

Our study demonstrated that; combined HCV therapy was associated with reduced total and free testosterone levels, which were restored by cessation of therapy. The assessment of interferon-induced changes in sex hormone serum levels has been so far limited to few studies (Barreca et al. 1993 and Corssmit et al. 2000). Our results are in consistent with Barreca et al. (1993) and Corssmit et al. (2000) who observed a significant decline of free and total testosterone serum concentrations but within the normal range over time. On the other hand; serum FSH and LH levels were not significantly affected during combined therapy course. Therefore; we assumed that the observed decrease in testosterone levels is not mediated by alteration of the pituitary–testicular axis. This, however, cannot be entirely excluded as gonadotropin secretion pulsatility was not analyzed.

In this study, serum prolactin levels were significantly increased during interferon therapy, however, its level did not significantly correlate with reduction in free testosterone serum concentrations (Kraus et al., 2005). This is in agreement with Genazzani et al. (1994) who reported the same results and observed that the duration and frequency

of prolactin secretory bursts from the pituitary are independent from gonadal steroid plasma levels in men. So the observed increase in serum prolactin values is not secondary to alterations in gonadal steroid concentrations. Rather we may speculate that interferon therapy either directly affects lactotrope cells in the pituitary, or hypothalamic factors which control prolactin secretion, or both.

In addition, platelets are considered as an important source of prothrombotic agents associated with inflammatory markers, and play a role in initiation and propagation of vascular and inflammatory diseases (Kilciler et al., 2010). Platelets with large size have many granules that can exert their hemostatic and proinflammatory actions with greater efficiency (Thompson et al., 1984). For this reasons MPV and PDW may be considered as indicators of platelet function and activation (Beyazit et al., 2012).

An increase in MPV has been observed in chronic viral hepatitis due to increase in the entrance of newly produced platelets into the circulation, which are larger in volume than the old platelets (Turhan et al., 2010).

Thrombocytopenia is a commonly detected haematological disorder during chronic HCV infection. Several mechanisms have been proposed as contributing to thrombocytopenia: sequestration of platelets in the enlarged spleen (Sanjo et al., 2003); platelet destruction mediated by platelet-associated IgG possibly leading to the sequestration in the reticuloendothelial system and also related to hypersplenism (Pockros et al., 2002); impaired hepatic production of thrombopoietin (Martin et al., 1997) and a direct viral effect since a positive correlation between thrombocytopenia and HCV association with platelets has been found (de Almeida et al., 2004).

Hepatitis C virus interacts with human platelets in vivo as a potential transport of infectious virions to the target liver. The binding of native viral particles with the platelet membrane glycoprotein VI (GPVI) was assessed. GPVI may be a platelet surface ligand for HCV playing a role in viral transport and persistence (Zahn et al., 2006).

Previous studies showed that; an increased trapping of platelets together with neutrophils and macrophages is shown in necrotic areas within the liver tissue. During the degranulation of theses platelets, transforming growth factor beta (TGF-β) and pro-fibrogennous mediators; platelet derived growth factor (PDGF) and epidermal growth factor (EGF) from alpha-granules of platelets will be released. This indicates that the disintegrated platelets can substantially participate in liver fibrogenesis. Also platelets, activated by these mediators, stimulate growth, the transformation of stellate cells and the synthesis of extracellular liver matrix (ECM) in stellate cells (Valkova, 2002).

In our study, multivariate analysis shows that platelet count may be considered as an important factor associated with SVR among patients who completed the treatment. This in in accordance with Everson et al.(2006) who reported that patients with low platelet counts ($\leq$125x $10^3/mm^3$) achieved lower SVR rates than patients with normal platelet counts (>125x $10^3/mm^3$) even in the case of patients with the same category of liver fibrosis treated by Peg-IFN plus ribavirin combination therapy. Thus, independent of liver fibrosis, thrombocytopenia itself seems to participate in treatment failure, although the mechanism remains unknown.

Moreover, in this study; MPV may be an essential factor associated with SVR among patients who completed the treatment. This finding is in agreement with Purnak et al.(2013) who reported that elevated MPV values in CHC at the time of diagnosis and in the post-treatment period may be an early sign of advanced liver fibrosis. We believe that two main mechanisms are responsible for increased MPV in CHC patients. One of them is inflammation, and the other one is secondary to chronic changes due to liver pathology in CHC as increased MPV may be the reflection of chronic microthrombosis in the portal bed in patients with advanced fibrosis through cascades of proinflammatory cytokines release in the course of disease progression (Beyazit et al., 2012).

In addition; although the liver is the primary site of metabolic clearance of circulating androgens; the vast majority of androgens are rather metabolized locally in target tissues (White et al., 2012). This could explain why circulating total testosterone levels were higher with increased fibrosis before the development of cirrhosis. In an experimental study; it had been proved that the HCV core protein augments androgen receptor (AR) signaling. The AR is a nuclear hormone receptor expressed in liver; its predominant ligand is testosterone and co-regulates numerous other genes such as inflammatory cytokines. HCV also enhances inflammatory response and release of interleukin-6 that activates STAT 3 phosphorylation which in turn activates AR (Kanda et al., 2008).

Moreover; in this work, lower levels of total testosterone as well as higher levels of free testosterone may be associated with SVR among patients who completed the treatment. This is in conjunction with White et al. (2012) who reported that circulating total testosterone levels were higher with increased fibrosis before the development of cirrhosis, with subsequently decreased SVR among these patients.

Previous study shows that prolactin plays an important role in the metabolic function of healthy liver and might play a role in the pathogenesis of liver cirrhosis as a regenerative factor which is not mediated by c-fos or c-jun (growth-associated genes like in the liver) (Brandenburg et al., 2005). It can be suggested from the current study that, lower levels of prolactin may be associated with SVR among patients who completed the treatment. This explanation may be due to that; prolactin induces the production of Interleukin-1 (IL-1) and interferon-γ, promotes the expression of IL-2 receptor, participates in the pathogenesis of autoimmune diseases stimulating anti-apoptotic activity and impairment of the negative selection of autoreactive B cells, and stimulates autoantibody production. In

systemic lupus erythematosus, prolactin seems to modulate IFN- γ secretion and to change the phenotype of dendritic cell from an antigen presenting cell to a pro-inflammatory cell (Shelly et al., 2012), which may affect the role of combined therapy and subsequently decreased SVR among these patients.

There are several limitations to the current study that must be taken in consideration; first, we didn't measure SHBG to assess the actual mechanism for elevated serum testosterone level. Second, our sample size was small and larger studies are needed to evaluate the effect of standard combined therapy on sex hormone levels and find the role of these hormones as predictors of SVR in the context of hepatitis C infection.

In conclusion, dysregulation of sex hormones can also occur in compensated liver disease in CHC patients. Higher total testosterone and lower free testosterone levels are associated with greater severity of liver disease measured by fibrosis score. Pegylated IFN-α treatment reduce total testosterone and free testosterone levels, but increase serum prolactin level with no changes in serum level of FSH and LH. Higher levels of platelet count and free testosterone levels as well as lower levels of total testosterone, MPV and prolactin levels may be good predictors of SVR. Thus, screening for thrombocytopenia, androgen deficiency and prolactin elevation in the context of hepatitis C infection should selectively target men with more severe liver disease or documented higher stages of fibrosis or presence of symptoms suggestive of androgen deficiency.

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Conflict of interest:

Authors declare no conflict of interest related to this article.

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