RESEARCH ARTICLE

Changes in Hematological Parameters with Pegylated Interferon in Chronic Hepatitis C Virus Infected Patients

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Abstract

The liver is one of the most common sites of cancer in the world, hepatocellular carcinoma (HCC) predominating. HCC is the sixth most common cancer and the third leading cause of cancer related death overall. Hepatitis C is a major risk factor and HCV is a rapid spreading virus which has become a problem globally, including in Pakistan. Interferon alpha therapy is used against HCV disease to regulate cell reproduction and to boost the immune system. In minute amounts interferon alpha is produced naturally by the immune system in HCV patients in response to hepatitis C virus and binds to receptors in the target cells and starts transcription of 20-30 genes due to which it develops an antiviral influence. Interferon is also administered artificially to overcome HCV disease and remove the biological effect of the virus from the infected site. The use of interferon or Peg-IFN plus Ribavirin treatment is also associated with adverse effects on body. For the current study, a convenient sample of 156 HCV positive patients of both males and females were taken. To collect blood CP and ALT, a reduction of level data and other important information were collected from the patients at regular intervals. Findings were 11.4 % in the red blood cells (RBC), 9.64 % in the total leukocyte count (WBC), 8.4 % in the hemoglobin levels (HB), 30.3 % in the platelet (Plt) count in both sexes. There was significant reduction in ALT levels due to Pegylated interferon plus ribavirin therapy. Hence strict haemotological monitoring of blood CP and ALT levels is necessary at regular intervals to reduce severe side effects which may lead to morbidity and mortality.

Keywords: HCV - pegylated interferon - ALT - ribavirin - heamtological parameters

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Introduction

Hepatocellular carcinoma (HCC) is the sixth most common tumor and the third leading cause of cancer-related deaths, particularly in Africa, China, Korea, and other Asian nations . The liver is one of the most common sites of cancer in the world, hepatocellular carcinoma (HCC) predominating (Makkoch et al., 2015). Transarterial chemoembolization (TACE), transarterial embolization (TAE), drug-eluting beads, and radioembolization have been used for locoregional control, and have been shown to prolong the overall survival when compared with supportive care (Kumar et al 2015).

Owing to excellent tumor-targeting, promised efficacy and favourable toxicity profile, the novel combination therapy of licartin and TACE could be applied in patients with unresectable HCC (Ma et al 2014). Combination therapy of pegylated interferon plus ribavirin has been commonly prescribed based on HCV infected patient body weight and specific genotypes. Pegylated interferon dose is administered once a week subcutaneously and ribavirin taken by chronic HCV infected patients in a dosage of 800 to1200 mg per day. The time duration of combination therapy is 6 months and may be extended to 12 months depending upon genotypes of hepatitis C virus (Ludovico et al., 2011). Combination treatment of Peg-IFN plus ribavirin is also known to have some side effects (Lagging et al., 2015). Though IFN occurs naturally in the body, however administering artificially may cause adverse effects. Either in1st or 2nd hours some side effects occur, while others may occur later. The appearance of these symptoms in 2-3 hours after the administration of drug is evident in more than half of HCV patients. The most common side effects at the starting days of the Peg-IFN plus ribavirin therapy are like influenza, chills, fever, muscle aches and pains, headache (Jacobson et al., 2011).

The other common side effects are changes in blood composition, i.e. RBC level, WBC level, HB count, Platelets count and ALT level which leads to serious disease like thrombocytopenia, anemia etc (Alam et al., 2015). These side effects of therapy negatively impact patient's quality of life. Other side effects with Interferon

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are heart and renal failure, hypo and hyperthyroidism, disease of auto immune and visual loss (Trakroo et al., 2015). Other serious side effects of this combination therapy are suicidal tendency, depression and irritability. Dysfunctions of thyroid can also be caused by this therapy. One of the most serious and rare side effects is the neuro-retinitis. At this stage the therapy is dangerous and thus stopped completely (Pouresmaeeli et al., 2015).

Interferon therapy is also observed to suppress the bone marrow (Cuerquis et al., 2014).Anemia is a common side effect in patients by ribavirin dose which causes hemolys is and leads to hemolytic anemia in taking combination pegylated interferon plus ribavirin treatment. At this point the treatment dosage needs to be adjusted or stopped altogether (Smolic et al., 2013).

In Pegylated interferon, pegylation works to extend its half-life and size for absorption. Interferon is removal from plasma is faster than pegylation of interferon,howeverdue to pegylation it works better. Hepatitis C patients having genotype 1 requires therapy for longer time because of lower response but the response result is higher when HCV patients are given therapy of pegylated interferon with ribavirin. The time duration of the therapy based on genotypes in HCV patients with genotype-2 and genotype-3 is 6 months, whereas the samples of HCV genotype-1 requires 1 year(National Institutes of Health, 2002; Pawlotsky et al., 1999).In Pakistan, Peg-INF is recommended for therapy of Hepatitis C patients by National Institute of Health (NIH). Interferon therapy of 3 MIU is given to the HCV positive patients three times a week for duration of 24 weeks (Alam et al., 2013)

Peg-INF plus Ribavirin therapy has been proved to have three folds efficiency to the HCV virological response (Masood et al., 2010).In most cases platelet count decrease due to therapy of pegylated interferon/ribavirin but in severe condition (Hezode et al., 2013) may leads to bleeding, at this stage the therapy needs to be stopped. IFN alpha therapy suppresses bone marrow which leads to cytopenia. In combination, IFN plus ribavirin therapy may causes anemia due to hemolysis. Ribavirin also cause anemia, because RBV enters in red blood cells and due to phosphorylation it is converted into active state, and that phosphorylation reduces adenosine triphosphate (ATP) which leads to ribavirin triphosphospahte (Maasoumy et al., 2013). Ribavirin triphosphospahte accumulates sixty fold than its normal concentration in plasma and it is difficult for erythrocytes to metabolize and results in damaging membrane oxidation and thus causing hemolysis. Erythropoiesis suppressed when regulation of erythrocyte receptor accumulates thus causing anemia. Haematological toxicities may be improvedby dose reduction however it carries the risk of an additional treatment response (Elshahawi et al., 2015).

Table 1. Age Groups of the HCV Positive Patients

Age Groups	Gender	
	Male	Female
Below 25 years	25	28
26-35 years	27	26
36 & Above	26	24
TOTAL	78	78

The aim of the present study was to identify the biochemical and hematological parameters of HCV patients taking the therapy of pegylated interferon plus ribavirin treatment with the following objectives, to investigate the effect of Peg-INF plus RBN therapy on ALT and on blood composition in chronically infected HCV patients.

Materials and Methods

The sample collection and study design was conducted at Shaheed Zulfikar Ali Bhutto Medical University (PIMS Hospital) Islamabad Pakistan from December 2014 to June 2015.Convenience sample of 156 HCV patients of both male and female were included in the study. The exclusion of patients from the study was made on the basis of alcoholic consumption or drug abuse, less controlled psychiatric disease, HBS Ag positive and liver disease at the final stage. Socio-demographic information i.e. age, sex, marital status, location, education, marital status, location and approach of patient knowledge with disease procedure like signs , causes, and symptoms etc. filled according to project questionnaire. Blood sampling from the HCV patients were carried out at the duration of one month up to the 3rd month of the treatment.

i). To confirm Hepatitis C viral RNA and Anti-sera; *ii*). Information about twenty four hours dietary recall; *iii*). Haemotological and biochemical tests; *iv*). Statistical analysis

Confirmation of Hepititis C Viral RNA And Antisera

Initially all the subjects were screened for anti-HCV antibodies by Immuno-Chromatographic Tests (ICT) and every HCV positive sample was tested twice then followed for anti-HCV antibodies by ELISA technique (Biokit, S.A, Barcelona-Spain) according to the instructions given by the manufacturer followed by RNA extraction and PCR confirmation of positive samples.

HCV RNA was extracted from 200 µl sample taken from serum by using Ana-gen RNA extraction Kit (Ana-gen, USA) followed by RT-PCR (reverse transcription PCR), by using of enzyme reverse transcriptase M-MLV (Fermentas, USA). The cDNA was prepared by their present primers, then amplified cDNA subjected for amplification of two round PCR. For PCR amplification initial denaturation was carried out at 95°C for 2 minutes followed by denaturation at 94°C by 45 seconds, annealing temperature of 54°C for 45 seconds and then extension at 72°C performed in a thermal cycler (Eppendorf, Germany) for one minutes. Inner primers of different set for the 2nd round PCR were used with the same conditions, however for first round amplification the annealing temperature was raised to 62°C.

The total products of PCR in first and second rounds were then visualized by agarose gel of 1.8% which was prepared in the TBE buffer of 0.5% and was stained with (10 mg/ml) ethedium bromide used as florescent dye. Alpha quant (Alpha Innotech) was used for gels photographing. For DNA size marker100-bp (Gibco BRL) DNA ladder was used.

Haemotological And Biochemical Tests

HCV positive patient's blood was collected and serum was separated from the blood samples by centrifugation technique. After that different hematological and biochemical results were carried out.

SGPT/ALT was also tested by Roche/Hitachi 902 which is a fully automatic analyzer and function on basis of spectrophotometer. Automatically the sample and reagents were mixed in a cuvette and SGPT/ALT was calculated at 340 nm wavelength. By automatic analyzer (Model XS-1000i) blood complete pictures was calculated. HCV patients WBC level, SGPT level, RBC level, HB count and platelet count were calculated at one month interval.

Statitical Analysis

The data generated was statistically analyzed by computer software program JMP. SAS (Version 7.0. SAS, USA).

Results

The study was carried out to analysis the changes in blood composition i.e. ALT, WBC, RBC, HB and Platelets count due to therapy of Peg-IFN plus ribavirin in HCV chronic patients. In the present study 156 samples were included. Both sexes were participated in the study comprising of 78 male and 78 female patients and were divided into three groups. Different blood levels were observed in chronic HCV subjects for 3 months interval (Table 1).

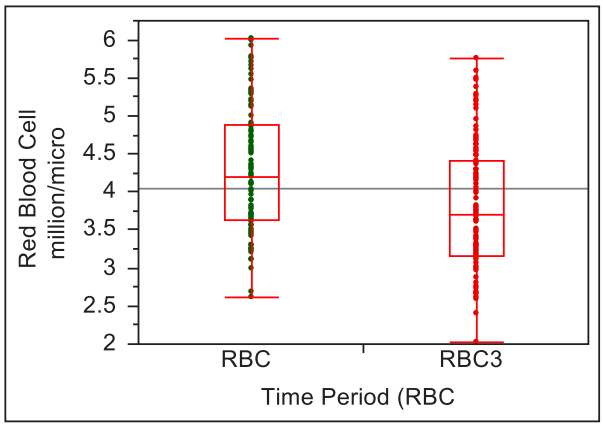

Figure 1. RBC Counts ($10^6/\mu$L)(n=156) at Baseline (before treatment) and after Treatment with Pegylated Interferon plus Ribavirin

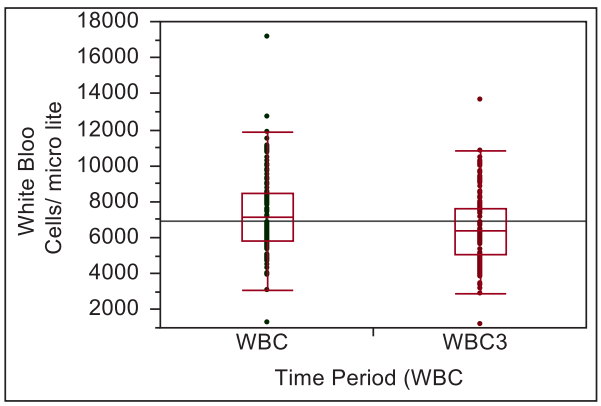

Figure 2. WBC Counts ($10^6/\mu$L)(n=156) at Baseline (before treatment) and after Treatment with Pegylated Interferon plus Ribavirin

Changes in Red Blood Cells

The changes in the mean blood composition of RBC levels in both sexes of chronic HCV patients taking pegylated interferon plus ribavirin therapy are shown in Figure.1. From the statistical analysis significant reduction ($p \leq 0.001$) was observed in RBC levels with a series of three months therapy. Mean changes in the RBC levels at the initial stage of treatment in males patients were 4.28 ± 1.45 (106/μL)and changes in the levels of RBC in females were 4.14 ± 1.29 (106/μL).According to baseline data of red blood cells levels in both male and female HCV chronic patients no significant difference was observed. Patients receiving Peg-IFN plus RBV therapy led to reduction of 11.04%red blood in both sexes having chronic HCV disease. In male patients 11.1% decrease and 11.0% decrease in females was observed (Figure 1).

Changes in White Blood Cells

The changes in the blood composition mean WBC levels in both sexes of chronic HCV patients taking pegylated interferon plus ribavirin therapy are showed in Figure.2. From the statistical analysis a significant reduction ($p \leq 0.001$) in WBC levels was observed with a series of three months therapy. In males changes in the levels of WBC (8324.36 ± 2757.72 (/μL) at the baseline vs. 7521.15± 1.81 at month 3) and changes in the levels of WBC in females were 8291.33 ± 2718.48 (/μL) was observed. According to baseline data of white blood cells in both male and female HCV chronic patients there was no significant difference. Receiving therapy of Peg-IFN plus RBV led to reduction of 10%in white blood cells in both sexes having chronic HCV disease. Thus in male patients9.9% decrease and 10.1 % decrease in females were observed (fig:2).

Changes in Hemoglobin

The changes in the blood composition mean HB count

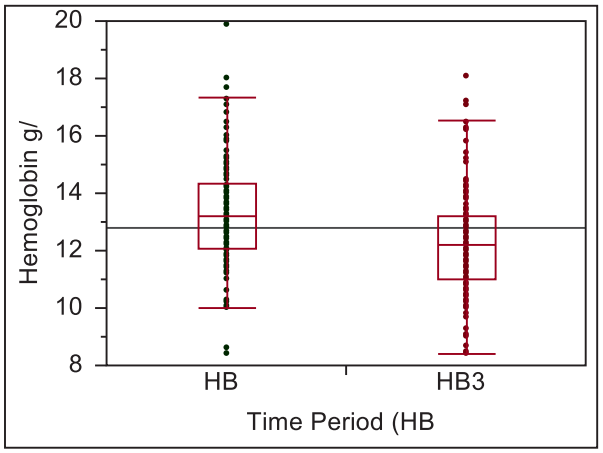

Figure 3. Hemoglobin Levels (g/dL) (n=156) at Baseline (before treatment) and after Treatment with Pegylated Interferon plus Ribavirin

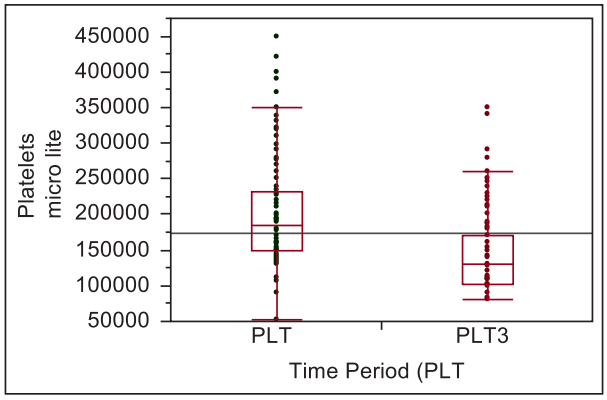

Figure 4. Platelet Levels (/μL) (n=156) at Baseline (before treatment) and after Treatment with Pegylated Interferon plus Ribavirin

in both sexes of chronic HCV patients taking pegylated interferon plus ribavirin therapy are showed in Figure.3. HB count was significantly reduced ($p \leq 0.001$) with a series of three months therapy. Mean changes in the levels of WBC at the initial stage of treatment in males patients were 13.35 ± 0.14 (g/dL) and changes in the levels of HB count in females were 12.55 ± 2.99(g/dL).According to baseline data of hemoglobin count in both male and female HCV chronic patients no significant difference was observed. Receiving therapy of Peg-IFN plus RBV led to reduction of 8.1% of HB in both sexes having chronic HCV disease. Thus in male patients8.25 % decrease and 7.96 % decrease in females were observed (fig:3).

Changes in Platemet Counts

The changes in the blood composition mean platelets count in both sexes of chronic HCV patients taking pegylated interferon plus ribavirin therapy are shown in Figure4. From the statistical analysis significant reduction ($p \leq 0.001$) in HB count with a series of three months therapy was observed. Mean changes in the levels of WBC at the initial stage of treatment in males patients were 208994.8 ± 49497.5 (/μL) and changes in the levels of PLT count in females were 201144.16 ± 484814.7 (/μL). According to baseline data of platelets count in both male and female HCV chronic patients there were no significant differences. Receiving therapy of Peg-IFN plus RBV led to reduction of 30.6% of PLT in both sexes having chronic HCV disease. Thus in male and female patients,30.7% and 30.5% decrease was observed respectively (fig:4).

Discussion

The major parameter that was studied in the research was changes in the blood composition in response to Hepatitis C infection. The changes in the blood composition were highly significant. For assessing blood composition changes, Red blood cells (RBC), Leucocyte Count (WBC), Hemoglobin (HB), Platelet (Plt) count were measured.

The changes were studied for three months interval during the pegylated interferon plus ribavirin therapy. Comparisons were also recorded between the changes after treatment data and baseline readings. The suitable treatment of chronic hepatitis C is the therapy of Peg-IFN alpha and RBN (Sugimoto et al., 2015).

The currently peg-interferon plus ribavirin therapy denoted as gold standard of therapy that improves responses for all genotypes (National Institutes of Health 2002; Hunyady et al., 2011). Peg-IFN alpha and ribavirin was administered to the patients for 6 months. Serum alanine aminotransferase (ALT) activity and variables were most commonly measured because it assess to hepatic disease (Nadeem et al., 2007). Due to large amount of ALT secretion into the blood caused liver injury.

In the present study all the patients (100%) receiving Combination Peg-IFN therapy showed uniform reduction in ALT levels and gradual normalization of the ALT levels. The uniform reduction in the ALT levels was found approximately same in the patients of both sexes. Similar findings have also found where no relationship was established between male and female and the response to therapy (Nadeem et al., 2007).

The total reduction in ALT levels in the present study is 29.62%. A 37.9% reduction (p=0.001) in ALT levels due to therapy is reported (Alam I et al., 2014). He also reported that 86% patients responded positively to combination Peg-IFN therapy, and 97% in the patients showed normalization at24 weeks of treatment. In the present study the response of the ALT to the therapy was positive.

In the present study all the HCV positive subjects (male and female) were taking Peg-IFN plus ribavirin therapy. Pegylated Interferon suppresses bone marrow as a result by thrombocytopenia and neutropenia. The TLC significantly (p=0.001) decreased in males and females. According to Iliescu, (2010) leukocytes (WBC) reduced 30-40% during therapy. In the reported study significant reduction in TLC of 1.11x103/μL (p=0.001) (Ghaniet al., 2009). The hematological disorders were reported during Peg-IFN and ribavirin therapy in chronic HCV patients.

RBC reduced by 21.13% which can lead to anemia. RBC is responsible for carrying oxygen and nutrients to the whole body. Its decrease in the blood can cause anemia. Low levels of RBC during interferon treatment, was also observed (Aspinall et al., 2004). In their study mostly HCV patients received pegylated interferon/ ribavirin combination treatment experience anemia in mild to moderate stage. In reported study the side effects in 11241 HCV positive patients treated with Peg-IFN alpha therapy. About 5 patients died due to liver failure because of interferon treatment. In eight positive patients adverse effects was life threatening. Two attempted suicide because of depression and six patients were reported with the bone marrow suppression. No hepatic diseases were observed in 131 positive patients, out of 131, the 71 patients having disease of thyroid, ten diabetes mellitus patients, five having auto-immune systemic disease, 5 with impotence, about 14 patients effected with the disease of dermatologic, ten with psychosis, seven with heart disease, four have seizures, three with neuropathy and two hemolytic anemia patients also included. The conclusion of this study was the adverse effects of interferon may be life threatening (Fattovich et al., 1996).

Total leukocyte count (TLC) reduced by1.30x109/L at

the therapy of three months. Decrease in TLC may lead to leucopenia. This is a hematological disease where the number of circulating WBCs decreases by a large amount. Leukocytes (WBC) are responsible for defense mechanism and provide immunity against infections in the body. When any of the sub-types of WBC reduced in a person, it leads to major risk of other diseases and infections. Other hematological parameters like Plt. Count, Hemoglobin also reduced significantly (p=0.001). HB levels in the patients were reduced by 8.4% in the current study. In a similar study conducted a significant fall of 2gm of mean Hemoglobin from base line at 6 months of treatment (p <0.001) was recorded (Ghani et al., 2009). Significant difference in HB count between both sexes was identified. Male showed 8.25% reduction while female showed 7.96 % reduction in Hb during therapy, in the last three months it tends to stabilized to its normal rate (Iliecu et al., 2010). Leukocytes (WBC) reduced 30-40% during therapy, with stabilization in 12 weeks and Platelet count levels decreased was more evident in HCV patients receiving pegylated interferon therapy than in those patients who used conventional interferon. To noticed downward trend in Platelets levels with decrease at23.19x109/mm3& 28.29/ mm3 of 12 & 24 weeks treatment (Saeed et al., 2006). Mean Plt. Count also decreased by 14.7% Plt. The results are also comparable with Ghani et al., 2009 in which Plt. Count decreased by 52x109/mm3 at 24 week of therapy. While in our study it is 30.3x109/mm3 at the end of 12th week of therapy.

The results of our recent study conducted to ascertain the hematological adverse effects of pegylated interferon antiviral therapy are comparable with the similar studies reported internationally. On the basis of our current data we strongly recommend to screen complete blood count (CBC) all chronic HCV patients receiving antiviral therapy for six month. In case of adverse side effects of such therapy, timely modification of dose or stopping of therapy may be lifesaving.

References

Alam I, Ali I, Ali S, et al (2013). Impact of combination interferon therapy on the body weight, body fat and lean body mass of chronic hcv infected patients. *J Antivir Anti retrovir*, **6**, 1-5.

Alam I, Hassan S, Alam I, et al (2015). PAIgG and PAIgM levels in secondary dengue virus infections lead to thrombocytopenia in patients from KP, Pakistan. *Asian Pac J Trop Biomed*, **5**, 801-5

Alam I, Alam I, Ali I, et al (2014). Weight loss in HCV patients can be used as surrogate marker for evaluation of interferon (IFN-α) treatment efficacy a prospective pilot study. *Pak J Pharm Sci*, **27**, 571-6.

Aspinall R, Pockros P (2004). The management of side effects during therapy for hepatitis C. *Alimentary Pharmacol Therapeutics*, **20**, 917-29.

Cuerquis J, Alam I, Marino IR, et al (2014). Human mesenchymal stromal cells transiently increase cytokine production by activated T cells before suppressing T-cell proliferation:effect of interferon-γ and tumor necrosis factor-α stimulation. C*ytotherapy*, **16**, 191-202.

Elshahawi Y, Sany D (2015). Microalbuminuria and pegylated interferon in hepatitis-C patients.*Saudi J Kidney Dis Transpl*, 26, 1183-9.

Fattovich G, Giustina G, Favarato S, et al (1996). A survey of adverse events in 11241 patients with chronic viral hepatitis treated with alfa interferon. *J Hepatol*, 24, 38-47.

Ghani MH, Masood N, Munir A, et al(2009). Haemotological disorders during interferon and ribavirin therapy in chronic hepatitis C patients. *Med Channel*, **15**, 167-70.

Hezode C, Fontaine H, Dorival C, et al (2013).Triple therapy in treatment-experienced patients with HCV-cirrhosis in a multicentre cohort of the French Early Access Programme. *J Hepatol*, **59**, 434–41.

Hunyady B, Kovács B (2011). Side-effects of interferon plus ribavirin therapy with or without protease inhibitor direct acting antiviral agents during treatment of chronic hepatitis C virus infection. *Orv Hetil*, **152**, 1997-09.

Ludovico A, Marta M, Piero L, et al (2011). The optimal dose of ribavirin for chronic hepatitis C. *Hepat Mon*, **11**, 240-6.

Lagging M, Brown A, Mantry PS (2015). Grazoprevir plus peginterferon and ribavirin in treatment-naive patients with hepatitis C virus genotype 1 infection: a randomized trial. *J Viral Hepat*, **23**, 80-8.

Iliecu G, Alam Z, White J, et al (2010). Haemotological monitoring of interferon based therapy for chronic viral hepatitis B and C. *Hepatology*. **14**, 1102-11.

Jacobson IM, Hutchison JG, Dusheiko G, et al (2011).Telaprevir for previouslyuntreated chronic hepatitis C virus infection. *N Engl J Med*, **364**, 2405-16.

Kumar, Y., Sharma, P., Bhatt, N et al (2015). Transarterial therapies for hepatocellular carcinoma: a comprehensive review with current updates and future directions. *Asian Pac J Cancer Prev*, **17**, 473-8.

Makkoch J, Praianantathavorn K, Sopipong W, et al (2015). Genetic variations in XRCC4 (rs1805377) and ATF6 (rs2070150) are not associated with hepatocellular carcinoma in Thai patients with hepatitis B virus infection. *Asian Pac J Cancer Prev*, **17**, 591-5.

Masood N, Hanif M, Munir A, et al (2010). End of treatment and biochemical response to interferon and ribavirin therapy in chronic hepatitis C patients. Med. Channel, 16, 219-222.

Maasoumy B, Port K, Serrano B, et al(2013).The clinical significance of drug-druginteractions in the era of direct-acting anti-viral agents against chronic hepatitis C. *Aliment Pharmacol Ther*, **38**, 1365-72.

Ma J, Wang J-H (2014). 131 I-Labeled-metuximab plus transarterial chemoembolization in combination therapy for unresectable hepatocellular carcinoma: results from a multicenter phase iv clinical study. *Asian Pac J Cancer Prev*, **16**, 7441-7.

Nadeem A, Aslam M, Hussain T, et al (2007). Efficacy of combined interferon alpha and ribavirin therapy in patients of chronic hepatitis C. *Pak J Physiol*, **3**, 32-40.

National Institutes of Health (2002). Consensus Development Conference Statement: Management of Hepatitis C, June 10–12.

Pawlotsky JM, Ali E, Jhon R, et al (1999). Diagnostic tests for hepatitis C. *J Hepatol*, 31, 71-9.

Pouresmaeeli M, Davila M, et al (2015). Efficacy and tolerability of peginterferonalpha-2a and peginterferon alpha-2b in Iranian patients with chronic hepatitis C. *Hepat Mon*, **15**, e30780.

Saeed K, Hussain T, Kamran M, et al (2006). Effect of antiviral therapy on haemotological parameters in patients with chronichepatitis. *Pak Armed Forces Med J*, **56**, 228-31.

Sugimoto K, Kim SR, Port K, et al (2015). Comparison of daclatasvir and asunaprevir for chronic HCV 1b infection with telaprevir and simeprevir pluspeginterferon and ribavirin, with a focus on the prevention of occurrence and

recurrence of hepatocellular carcinoma. *Oncology*, **2**, 42-46.
Smolić M, Cho SH (2013). Management of side effects induced by antiviral therapy for chronic hepatitis infection. *Acta Med Croatica*, **67**, 383-7.
Trakroo S, Qureshi K (2015). Successful treatment of chronic hepatitis c infection with direct-acting antivirals in a heart transplant recipient: a case report. *Transplant Proc*, **47**, 2295-7.