Interferon Antibody in Predicting the Response to Interferon Therapy in HCV Patients

Mousa A. Hussein[1], Naser K. Elhamshary[2], Mosaad M.Ibrahim[3] and Ibrahim H. Mohammed[3]

Departments of Internal Medicine[1], Tropical Medicine[2] and Medical Biochemistry[3]
The International Islamic Center for Population Studies and Research-ART unit, Faculty of Medicine, Al-Azhar University, Egypt.
elfaroklab@yahoo.com; ahmed.nasser2257@yahoo.com

Abstract: Introduction: Different mechanisms have been proposed for the failure of interferon (IFN) therapy in patients with chronic hepatitis C, for example, the presence of IFN-neutralizing antibodies. So the present study was performed to see the frequency of formation of interferon antibodies and other factors in patients receiving alpha interferon and evaluate their role in treatment response. **Methods:** the study included 100 patients with chronic Hepatitis C receiving pegylated interferon alpha 2a and ribavirin in Al-Obour Insurance outclinic (kafr elsheikh governorate), from January 2009 to April 2012. Sera were collected from all patients before and after 12 weeks from the start of interferon therapy and analyzed for interferon antibody. **Results:** After 3 months of treatment 68 patients were respond to interferon therapy proofed by negative PCR for HCV RNA, most of them were males (41/27, 60.3%) and younger ages (group I), while 32 patients still had positive PCR for HCV, they were older ages and more females (group II). Antibody levels of over 20 U/ml should be taken as technical cut off values. Majority of the cases (72) had antibody levels of less than 20 U/ml, 7 of them non-responder. (18) cases having values ranging between (20-50 U/ml), 15 of them non-responder. Only (10) cases had values over 50 U/ml, all of them non-responder to IFN therapy. Serum levels of interferon-Ab were significantly higher in group II when compared to group I. Before treatment BMI, levels of viremia stages of liver fibrosis and activity were significantly higher in group II than group I. After 3 months of treatment serum levels of interferon-Ab become above the cutoff levels of significantly and very highly significantly elevated (58.5±5.8 U/ml) more than group I (18.5±5.1 U/ml), which still below the detection limit of its levels, also WBC were significantly decreased in group I when compared to group II. Increases virological response were associated with low serum levels of interferon-Ab, male sex, younger patients, low BMI, high leucocytes count before treatment that become low during treatment, low grade of fibrosis and activity and low Viremia, but not associated with serum cholesterol. Logistic regression analysis confirmed that the most predictors of virological response in patients with HCV under interferon therapy were low serum levels of interferon-Ab, younger ages, male sex, BMI, WBC, metaver score and viremia. **Conclusions:** Interferon antibodies are formed in a variable percentage of cases receiving interferon that may be affect interferon therapy. Low levels of interferon-Ab before treatment that increased during treatment are a predictive for virological response. Serial antibody levels may be done to see if they remain stationary or increase with the continuation of the therapy.

[Mousa A. Hussein, Naser K. Elhamshary, Mosaad M.Ibrahim and Ibrahim H. Mohammed **Interferon Antibody in Predicting the Response to Interferon Therapy in HCV Patients**. *Nat Sci* 2013;11(4):104-109]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 17

Keyword: Interferon Antibody, Predicting, Response, Interferon, Therapy, HCV Patients.

1. Introduction

Chronic hepatitis C virus (HCV) infection is one of the major causes of chronic liver disease worldwide. The treatment of chronic hepatitis C is based currently on a combination of pegylated interferon alpha (PEG-IFN-α) and ribavirin leading to viral eradication in about 50% of treatment-naive patients infected with genotype 1 [**Poynard *et al.*, 2003].** It is well known that treatment with IFN-α leads to the development of neutralizing anti-IFN antibodies in some patients. However, the contribution of anti-IFN antibodies to IFN non-responsiveness in chronic HCV infection is still a matter of debate **[Antonelli *et al.*, 1996].**

Interferon (IFN) antibodies are produced during treatment for viral or neoplastic diseases. Natural antibodies to IFN have been found in the serum of normal individuals **(Caruso *et al.*, 1990**) and patients with autoimmune diseases (**Meager *et al.*, 1997**) viral infections **(Ikeda *et al.*, 1991**) or tumours **(Trown *et al.*, 1983**) but development of antibodies is pronounced in those receiving exogenous interferon. Both neutralizing and non neutralizing (binding) antibodies have been detected but neutralizing antibodies are associated with failure of IFN therapy. Binding antibodies prevent normal clearance and degradation of administered IFN alpha **(Rosenblum *et al.*, 1985**). Though these antibodies appear in both responders and non responders but they appear much earlier in non responders than in responders **(Milella *et al.*, 1993).**

The present study was performed to investigate the development of anti-IFN antibodies in patients with HCV chronic hepatitis treated with IFN-α s and to analyze the influence of these antibodies on treatment outcomes.

2. Patients and Methods

One hundred patients (56 males and 44 females) with chronic HCV related liver disease referred to Al-Obor Insurance out clinic from May 2010 to April 2012 were concluded in our study. The diagnosis of HCV depended on elevation of serum transaminases, positivity of HCV-ab, positive PCR and liver biopsy.

Patients were subjected to the following; Complete history and clinical examination, with special emphasis to hepatic manifestations of chronic liver disease, such as chronic fatigue, malaise, loss of weight, anorexia, peritibial edema and jaundice. Patients chronically infected with HCV, who were treated with pegylated interferon α-2a (1.5 μg per kilogram body weight, once weekly, subcutaneously) and ribavirin (600-800 mg daily). Sera were collected and stored at -80°C until ready for use. Sera were collected from all patients before and after 12 weeks from the start of interferon therapy and analyzed for interferon antibody.

Measurements of HCV-Ab, HBS-Ag (those with HBS-Ag excluded from the study) and quantitative PCR for HCV–RNA. We investigated interferon alpha antibody using ELISA (Enzyme-linked immunosorbent assay) (**Antonio *et al.*, 2004) (TECO DIAGNOSTICS)**. In addition to evaluated of CBC, prothrombin time, serum albumin, bilirubin, ALT, AST, serum iron, ANA, blood urea and creatinine, total cholesterol, fasting blood sugar, (TSH, alpha feto protein using ELFA technique (Enzyme linked Fluorescent assay) (VIDAS BIOMERIEUX)) to determine their eligibility for antiviral treatment.

Inclusion criteria:

- 18 to 60 years of age both sex, Chronic genotype 4 HCV infection
- Treatment Naive sero-negative for HBV and HIV viral infections
- Absolute neutrophil count of >1500 per cubic millimeter
- A platelet count of >80,000 per cubic millimeter with normal hemoglobin level

Exclusion criteria:

- Patients with decompensate liver cirrhosis (consider for liver transplantation).
- Evidence of primary hepatocellular carcinoma and organs transplantation
- Patients with biliary, cardiac and metabolic cirrhosis
- Low CBC count before treatment
- Patients with evidence of another cause for chronic hepatitis such as alcohol excess or auto–immune liver disease (smooth muscle antibody positive, high IgG) and co-infected with the hepatitis B virus
- Patients with co–morbidity due to neoplasia, cardiac, respiratory or renal disease, immunological mediated diseases and significantly retinal abnormalities
- Patients unlikely to be able to co–operate with subcutaneous injections or follow–up at the clinic
- Patients with unstable and severe mental health problems
- Pregnancy, breast–feeding mother, or pre–menopausal female not using effective contraception
- Patients with contraindications to use of PEG–IFN or ribavirin such as uncontrolled diabetes and epilepsy or compromised CNS function, significant depression, congestive cardiac failure, renal failure, psoriasis or known hypersensitivity to either product
- Patients with history of antiviral therapy for HCV in the previous 6 month.

Ultrasound guided liver biopsy were performed to determine grade of necroinflammation according to Metavir scoring system **(Poynard *et al.*, 1997).**

Statistical analysis:

Statistical analysis of the results was performed using an X2 test. Data were expressed as Mean ± SD. Differences between groups were tested with tailed-student's t-test for unpaired data. A value of $P \leq 0.05$ and a value of $r \geq 0.05$ were considered significant. Our results confirmed that Interferon antibodies are formed in a variable percentage of cases receiving interferon that may be affect interferon therapy and low levels of interferon-Ab before treatment that increased during treatment is a predictive for good virological response.

3. Results

After 3 months of treatment 68 patients were respond to interferon therapy proofed by negative PCR for HCV, most of them were males (41/27, 60.3%) and younger ages ((group I), while 32 patients still had positive PCR for HCV, they were older ages and more females (group II). According to the leaflet enclosed with the kit, antibody levels over 20 U/ml should be taken as technical cut off values. Majority of the cases (72) had antibody levels of less

than 20 U/ml, 7 of them non-responder. (18) cases having values ranging between (20-50) U/ml, 15 of them non-responder. Only (10) cases had values over 50U/ml, all of them non-responder to IFN therapy.
Serum levels of interferon-Ab were significantly higher in group II when compared to group I. Before treatment BMI, levels of viremia stages of liver fibrosis and activity were significantly higher in group II than group I (Table 1).

After 3 months of treatment serum levels of interferon-Ab become above the cutoff levels of significantly and very highly significantly elevated (58.5±5.8 U/ml) more than group I (18.5±5.1 U/ml), which still below the detection limit of its levels, also WBC were significantly decreased in group I when compared to group II (Table 2).

Increases virological respond were associated with low serum levels of interferon-Abs, male sex, younger patients, low BMI, high leucocytes count before treatment that become low during treatment, low grade of fibrosis and activity and low Viremia, but not associated with serum cholesterol.

Logistic regression analysis confirmed that the most predictors of virological response in patients with HCV under interferon therapy were low serum levels of interferon-Ab (OR 6.85, 95% CI 2.8-6.7, p<0.001), younger ages (OR 4.24, 95%CI 2.7-5.7, p <0.01), male sex (OR 3.53, CI 1.9-4.2, p <0.05), BMI (OR 3.45, CI 1.8-4.1, p <0.05), WBC (OR 3.24, CI 1.6-4.0, p <0.05), metaver score (OR 2.83, CI 1.5-3.7, p <0.05) and viremia (OR 2.42, CI 1.3-3.2), Table 3.

Table (1): Clinical and laboratory characteristics of the subjects before treatment.

Groups / Parameters	Group (1) respond to treatment after 12 weeks (n=68)	Group (II) not respond to treatment after 12 weeks (n=32)	*p*-value
Age (years)	45.5±6.7	52.8±6.4	<0.01
Sex M/F)	41/27	15/17	<0.05
ALT (U/L)	85.5±8.9	88.9±9.2	NS
AST (U/L)	82.9±10.8	83.5±11.4	NS
Bilirubin (mg/dl)	0.9±0.2	0.85±0.3	NS
Albumin (gm/dl)	4.2±0.8	4.1±0.6	NS
Prothrombin Concentration%	85±5	84±5.2	NS
TSH (µIU/L)	2.4±0.5	2.1±0.6	NS
Hb (gm/L)	13.9±0.8	12.8±0.5	NS
Platelet count (10^3/ml)	168±25	145±13	NS
WBC (10^3/ml)	5.8±0.4	5.6±0.6	NS
Interferon-Ab (U/ml)	12.5±5.1	16.5±5.8	<0.05 (S)
PCR for HCV-RNA	$4.6x10^6$±$0.8x10^3$	$6.5x10^6$±$0.5x10^3$	<0.05 (S)
BMI	25±2.1	28.8±1.8	<0.05 (S)

Table (2): Clinical and laboratory characteristics of the subjects after 3 months of treatment.

Groups / Parameters	Group (1) respond to treatment after 12 weeks (n=68)	Group (II) not respond to treatment after 12 weeks (n=32)	*p*-value
ALT (U/L)	35.5±3.5	38.9±3.2	NS
AST (U/L)	32.9±8.5	83.5±6.5	NS
Bilirubin (mg/dl)	0.8±0.2	0.75±0.3	NS
Albumin (G/dl)	4.3±0.7	4.1±0.8	NS
Prothrombin Concentration%	90±5	85±5.2	NS
TSH (µIU/L)	2.2±0.3	2.3±0.5	NS
Hb (gm/L)	11.9±0.9	10.8±0.6	NS
Platelet count (10^3/ml)	145±22	134±9	NS
WBC (10^3/ml)	3.8±0.4	4.6±0.6	<0.05(S)
Interferon-Ab (U/ml)	18.5±5.1	58.5±5.8	<0.05 (S)

Table (3): Logistic regression analysis for predictor factors of viral responded after 3 months of treatment.

Parameters / Variables	OR	95% CI	*p*-value
Interferon -Ab	6.85	2.8-6.7	<0.01 (HS)
Age	4.24	2.7-5.7	<0.01(HS)
Male sex	3.53	1.9-4.2	<0.05(S)
BMI	3.45	1.8-4.1	<0.05(S)
WBC	3.24	1.6-4.0	<0.05(S)
Metaver score	2.83	1.5-3.7	<0.05(S)
Viremia	2.42	1.3-3.2	<0.05(S)

4. Discussion

The role of neutralizing antibodies (NAb) in determining responses to antiviral therapy has not been defined well. In the present study, we have established an experimental system to measure NAb titers using ELISA method and measured NAb titers in patients with chronic hepatitis C who were treated with PEG-IFN/RBV.

Our study reported that majority of the cases (72) had antibody levels of less than 20, 7 of them non-responder. 18 cases having values ranging between 20-50, 15 of them non-responder. Only 10 cases had values over 50, all of them non-responder to IFN therapy.

The association between anti-IFN antibodies and non-response to IFN therapy is still controversial. Correlations between antibody formation and non-response, relapse and breakthrough have been reported in some studies **[Milella *et al.*, 1993; Bonetti *et al.*, 1994; Giannelli *et al.*, 1994; Roffi *et al.*, 1995; Antonelli *et al.*, 1996; Leroy *et al.*, 1998**], but not in others **[Craxi *et al.*, 1988; Bonino *et al.*, 1997; Hou *et al.*, 2000].** This discrepancy may be explained by differences in IFN preparations used, dosage or route of administration.

The time point of antibody development also seems to be crucial, anti-IFN antibodies that develop late during therapy probably have no impact on treatment response **[Giannelli *et al.*, 1994; Hou *et al.*, 2000].** Furthermore, previous studies suggested that the frequency of anti- IFN antibodies increases with duration of IFN therapy [**Steis *et al.*, 1988; Giannelli *et al.*, 1994].** There is however no data available from larger studies regarding the development of neutralizing antibodies in patients who did not respond to anti-viral therapy.

In Pakistan a six month therapy of 3 million units three times a week is recommended, which is a low dose and short course, while the highest dose of alpha interferon is pegylated interferon 180 micograms every week for 6-12 months and even with this dose antibodies have been infrequently reported (**Zuberi and Arif, 2002).** Interferon antibodies are more frequently formed in those receiving beta interferon Vs those getting alpha, but even in those receiving alpha interferon it is generally those who are either taking a high dose of interferon or are taking the drug for a long time like in mycelia or multiple sclerosis. However in liver disease usually alpha interferon is given and also in a low dose and in most cases therapy does not extend beyond 12 months **(Leroy *et al.*, 1998).**

Huma *et al.* (2007) shows that antibody production with alpha interferon in patients receiving treatment for chronic HCV infection is low and is not hampering with the treatment response. In this study single blood sample was taken from each patient any time after 3 months of initiation of the therapy, therefore it is possible that some cases might have been under reported as that might have produced low level antibodies at that time. It would be worthwhile to collect serial samples in few cases and see if values go up each month or are stable irrespective of the duration of therapy.

In contrast to our results **Mikiko *et al.* (2010)** reported that interferon antibodies were more frequently detected in sera of patients who achieved EVR (early virological response) compared to Non-EVR. They demonstrated that interferon-Ab titers in the pre-treatment patients' sera were associated with the good responses (EVR and SVR) to PEG-IFN/RBV combination therapy. Also it was reported that NAb titers to HCV-LPs were higher in patients who achieved an SVR with IFN/RBV therapy than in relapsers and non-responders (**Baumert *et al.*, 2000).** The better humoral responses, such as NAb, might be associated with better cell mediated immune responses, which involve CD4+ and CD8+ T cells. It is well known that peripheral and intrahepatic CD8+ T cell responses, which play an important role in the control of and recovery from HCV infection, are also important in determining an SVR in response to PEG-IFN/RBV treatment (**Caetano *et al.*, 2008).**

We **Mikiko *et al.*(2010)** found that NAb titers did not decrease significantly when measured even one year after disappearance of HCV RNA in the serum (Another important finding in this study is that the NAb in patients infected with HCV-1b

significantly cross-reacts to HCV-2a; they observed that average NAb titers of HCV-2a-infected patients were ca. 3 times higher than those of HCV-1b-infected patients when measured with the same experimental system using the J6/JFH1 strain of HCV-2a (439±2.72 vs. 139±2.48; $p<0.0001$). This information would be helpful when considering immunological prophylaxis against HCV infection, either active or passive immunizations using vaccines and NAb.

Both viral and host factors play important roles in the control of viral infection. Whereas viral factors help to adjust the cellular environment to support viral replication, host factors generally function to combat the viral invasion either by actively blocking the virus replication through innate and/or acquired immune responses or by having the infected cells die out by themselves through apoptosis so that the virus can no longer replicate in the infected cells **(Mikiko *et al.*, 2010).**

Acquired immune responses of the host involve cell-mediated immunity and humoral immunity. The importance of cellular immunity in combating HCV infection has been well documented (**Bungyoku *et al.*, 2009)**. On the other hand, humoral immune responses in protection against and/or recovery from HCV infection may be of less importance. Nevertheless, it has been reported that the neutralizing antibody (NAb) responses play an important role in the prevention of infection and in limiting viremia (**Youn *et al.*, 2005**). Indeed, patients chronically infected with HCV were reported to possess relatively high titers of cross-reactive NAb (**Bartosch *et al.*, 2003**).

It is reported that patients with chronic hepatitis C infection also have high NAb titers to envelope protein of HCV-like particles (HCV-LPs) (**Baumert *et al.*, 2000)**. Humoral and cellular immune responses are also important in determining response to antiviral therapy with IFN/RBV (**Cramp *et al.*, 2000).**

It is previously reported that the degree of antibody responses to the NS5A protein of HCV was correlated with early virological response after the initiation of PEG-IFN/RBV therapy **El-Shamy *et al.*, 2007).** However, the role for NAb in determining responses to PEG-IFN/RBV antiviral therapy has not been well documented.

Our result reported that the main factors related to good virological respond are low serum levels of interferon-Ab, younger ages, male sex, BMI, WBC, metaver score and viremia. However **Poynard *et al.* (2003)** Reported that the main factors for a successful treatment of chronic hepatitis C are a short duration of disease, young age at infection, female sex, low HCV serum levels, normal body weight, low alcohol consumption, infection by HCV genotype 2 or 3, and absence of neutralizing anti-IFN antibodies

Conclusions: Interferon antibodies are formed in a variable percentage of cases receiving interferon that may be affect interferon therapy. Low levels of interferon-Ab before treatment that increased during treatment are a predictive for virological response. Serial antibody levels may be done to see if they remain stationary or increase with the continuation of the therapy.

References

1- Antonelli G, Giannelli G, Currenti M, Simeoni E, Del Vecchio S, Maggi F, Pistello M, Roffi L, Pastore G, Chemello L and Dianzani F. (1996): Antibodies to interferon (IFN) in hepatitis C patients relapsing while continuing recombinant IFN-alpha2 therapy. Clin Exp Immunol 104:384–387.

2- Antonio AB, Rose ABT, Fátima MT, Norma de PC and Bruno AC. (2004): Are Anti-Interferon Antibodies the Cause of Failure in Chronic HCV Hepatitis Treatment? The Brazilian Journal of Infectious Diseases; 8 (1):10-17.

3- Bartosch B, Bukh J, Meunier JC, Granier C, Engle RE, Blackwelder WC, Emerson SU, Cosset FL and Purcell RH. (2003):. *In vitro* assay for neutralizing antibody to hepatitis C virus: evidence for broadly conserved neutralization epitopes. Proc Natl Acad Sci U S A. 100: 14199-14204.

4- Baumert TF, Wellnitz S, Aono S, Satoi J and Liang TJ. (2000): Antibodies against hepatitis C virus-like particles and viral clearance in acute and chronic hepatitis C. Hepatology 32: 610-617.

5- Bonetti P, Diodati G, Drago C, Casarin C, Scaccabarozzi S, Realdi G, Ruol A and Alberti A. (1994): Interferon antibodies in patients with chronic hepatitic C virus infection treated with recombinant interferon alpha-2 alpha. J Hepatol 20:416–420.

6- Bonino F, Baldi M, Negro F, Oliveri F, Colombatto P, Bellati G and Brunetto M. (1997): Clinical relevance of anti-interferon antibodies in the serum of chronic hepatitis C patients treated with interferonalpha. J Interferon Cytokine Res 17:S35–38.

7- Bungyoku Y, Shoji I and Makine T (2009): Efficient protection of infectious hepatitis C virus with adaptive mutations in cultured hepatoma cells. J Gen Virol. 90: 1681-91.

8- Caetano J, Martinho A, Paiva A, Pais B, Valente C and Luxo C. (2008): Differences in hepatitis C virus (HCV)-specific CD8 T-cell phenotype during pegylated alpha interferon and ribavirin treatment are related to response to antiviral therapy in patients chronically infected with HCV. J Virol. 82: 7567-77.

9- Caruso A, Bonfanti C, Colombrioita D, de Francesco H, De Rango C, Foeisti J *et al.* (1990): Natural antibodies to IFN-gamma in man and their increase during viral infection. J Immunol; 144:685-90.
10- Cramp ME, Rossol S, Chokshi S, Carucci P, Williams R and Naoumov NV. (2000): Hepatitis C virus-specific T-cell reactivity during interferon and ribavirin treatment in chronic hepatitis C. Gastroenterology. 118: 346-55.
11- Craxi A, DiMarco V, Volpes R and Palazzo U. (1988): Anti-alpha interferon antibodies after alpha interferon treatment in patients with chronic viral hepatitis. Hepatogastroenterology 35:304–305.
12- El-Shamy A, Sasayama M, Nagano-Fujii M, Sasase N, Imoto S, Kim SR and Hotta H. (2007): Prediction of efficient virological response to pegylated interferon/ribavirin combination therapy by NS5A sequences of hepatitis C virus and anti-NS5A antibodies in pre-treatment sera. Microbiol Immunol. 51: 471-82.
13- Giannelli G, Antonelli G, Fera G, Del Vecchio S, Riva E, Broccia C and Schiraldi O and Dianzani F. (1994): Biological and clinical significance of neutralizing and binding antibodies to interferon-alpha (IFNalpha) during therapy for chronic hepatitis C. Clin Exp Immunol 97:4–9.
14- Hou C, Chuang W, Yu M, Dai C, Chen S, Lin Z, HsiehM,WangL, Tsai J and Chang W. (2000): Incidence and associated factors of neutralizing anti-interferon antibodies among chronic hepatitis C patients treated with interferon in Taiwan. Scand J Gastroenterol 35:1288–1293.
15- Huma Q, Ambreen A, Waquaruddin A, Syed EA, Syed AM, Najib H and Saleem H. (2007): Role of interferon anti body in predicting the response to interferon therapy in HCV patients. JPMA 57:581-583.
16- Ikeda Y, Toda G, Hashimoto N, Umeda N, Miyake K and Yamanaka M (1991): Naturally occuring anti-interferon alpha-2a antibodies in patients with acute viral hepatitis. Clin Exp. Immunol; 85:80-4.
17- Leroy V, Baud M and de Traversay C (1998): Role of anti-interferon antibodies in breakthrough occurrence during alpha 2a and 2b therapy in patients with chronic hepatits C. J Hepatol; 28:375-381.
18- Meager A, Vincent A, Newsom-Davis J and Willcox N. (1997): Spontaneous neutralizing antibodies to interferon-alpha and interleukin-12 in thymoma-associated autoimmune disease. Lancet; 350:1596-7.
19- Mikiko S, Lin D, Soo R, Yoshi-hiro I, Ikuo S and Hak H. (2010): Analysis of Neutralizing Antibodies against Hepatitis C Virus in Patients Who Were Treated with Pegylated-Interferon *plus* Ribavirin. Kobe J. Med. Sci., Vol. 56, No. 2, pp. E60-E66.
20- Milella M, Antonelli G, Santantonio T, Correnti M, Monno L and Mariano N (1993): Neuterizing antibodies to recombinant alpha-interferon and response to therapy in chronic hepatitis C virus infection. Liver; 13:146-50.
21- Poynard T, Bedossa P, Opolon P for the OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups (1997): Natural history of liver fibrosis progression in patients with chronic hepatitis C. Lancet, 349:825-832.
22- Poynard T, Yuen M, Ratziu V and Lai C. (2003): Viral hepatitis C. Lancet 362:2095–2100.
23- Rosenblum MG, Unger BW, Gutterman JU, Hersh EM, David GS and Frincke JM (1985): Modification of human leukocyte interferon pharmacology with monoclonal antibody. Cancer Res; 45:2421-4.
24- Roffi L, Mels G, Antonelli G, BellatiG, Panizzuti F, Piperno A, Pozzi M, Ravizza D, Angeli G and Dianzani F (1995): Breakthrough during recombinant interferon alfa therapy in patients with chronic hepatitis C virus infection: Prevalence, etiology, and management. Hepatology 21:645–649.
25- Steis R, Smith J, Urba W, Clark J, Itri L, Evans L, Schoenberger C and Longo D (1988): Resistance to recombinant interferon alfa-2a in hairy-cell leukemia associated with neutralizing anti-interferon antibodies. N Engl J Med 318: 1409–1413.
26- Trown PW, Kramer MJ, Dennin RA Jr, Comell EV, Palleroni AV and Quesada J (1983): Antibodies to human leukocyte interferons in cancer patients. Lancet; 1:81-4.
27- Youn JW, Park SH., Lavillette D, Cosset FL, Yang SH., Lee CG, Jin HT, Kim CM, Shata MT, Lee DH, Pfahler W, Prince AM and Sung YC. (2005): Sustained E2 antibody response correlates with reduced peak viremia after hepatitis C virus infection in the chimpanzee. Hepatology. 42: 1429-36.
28- Zuberi SJ and Arif A. (2002): Serotyping of hepatitis C in Pakistan. J Pak Med Assoc; 52:218-219.

2/2/2013