ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Fas and Fas-ligand as markers for response to Interferon plus Ribavirin therapy in Egyptian patients with Chronic Hepatitis C

Amal A. Mohamed[1], Naglaa EL-Toukhy[2], Ahmed Ebrahim[3], Doaa Mohamed[4], Asmaa kamal[5], Naglaa F. Ghoname [6], Manal Mohamoud[7]

1. Biochemistry Department, National Hepatology & Tropical Medicine Research Institute, Cairo, Egypt.
2. Hepatology&Gastroentology and Infectious diseases Department, Faculty of medicine, Benha University, Egypt.
3. Biochemistry Department, Faculty of Science, Cairo University, Egypt.
4. Biochemistry Department, Armed Forces Central lab, Cairo, Egypt.
5. Department of Clinical & Chemical pathology, Faculty of medicine, Cairo University, Egypt.
6. Microbiology & Immunology Department, Faculty of Medicine, Tanta University, Egypt.
7. Internal Medicine Department, Faculty of Medicine, Ain Shams University, Egypt.

Manuscript Info

Manuscript History:

Received: 19 November 2013
Final Accepted: 20 December 2013
Published Online: January 2014

Key words:
Hepatitis C, Pegylated interferon, SVR, Fas, FasL.

***Corresponding Author**

Dr Amal Ahmed Mohamed

Abstract

Background/Aim: Egypt has the highest prevalence of hepatitis C virus (HCV) in the world. The role of apoptosis in treatment-induced HCV clearance is controversial. Recent studies have indicated that serum apoptosis-related cytokines (Fas and Fas ligand) may be one of the factors that used for evaluating the response to combination therapy in chronic HCV patients.

Methods: Serum levels of soluble Fas (sFas) and soluble Fas ligand (sFasL) were measured by ELISA in 50 naive patients with chronic HCV treated with combination therapy at baseline and at the end of treatment.

Results: Baseline sFasL level was significantly higher in responders as compared to non responders (p=0.000). After treatment, both sFas and sFasL levels were increased in responders. High baseline sFasL was the only predictor for response with a cutoff level 156.5 pg/ml gives an excellent prediction for both end of treatment response and sustained virological response with an area under the curve (AUC) of 86.0% and 85.4% respectively.

Conclusion: Virological response during HCV therapy was associated with an increase of sFas and sFasL. sFasL seems to predict which patients will achieve response to treatment.

Introduction

Hepatitis C virus infection (HCV) is recognized as a major health problem, its worldwide prevalence has been estimated to be ranging from 2.2% to 3.0% (Lavanchy, 2009). In addition, hepatitis C infection is a leading cause of chronicity, estimated to be more than 85% and is the main cause of developing decompensated liver cirrhosis and hepatocellular carcinoma (HCC) (Cornberg et al., 2011). The immune response stimulated by hepatitis C virus infection, its exact mechanisms and its role in disease progression and response to treatment with antiviral therapy are still not completely understood and are still needing to be clarified (Darwish et al., 2012).

The cytokine response to HCV infection could be of great importance in the host defense mechanism against HCV (Martin et al., 1999). Fas and Fas ligand apoptosis-mediated cytokines are reported to be involved in the HCV

pathogenesis and in the elimination process of the virus (Okazaki et al., 1996; Lio et al., 1998; Hayashi and Mita, 1999).

Fas is a glycosylated cell-surface protein excreted in various tissues, in particular in the liver, and inactivated mature lymphocytes or virus-transformed lymphocytes (Guicciardi et al., 2010). Soluble Fas (sFas) is produced as a result of shedding of the Fas extracellular portion or by alternative splicing of the transcripts (Cascino et al., 1995; Papoff et al., 1996). Fas ligand (FasL) is a transmembrane protein, mainly expressed on the cell surface of activated T cell. In the liver, the interaction between FasL- positive cytotoxic T lymphocytes and target tells, such as virus- infected cells or cancer cells, which usually overexpress Fas, represents a powerful tool to eliminate potentially toxic cells (Guicciardi and Gores, 2005). In chronic HCV infection, soluble FasL (sFasL) may be generated after proteolysis of the native and functional FasL and presents a pro-apoptotic capacity (Gruss, 1996). Recently, the roles of sFas and sFasL, have been evaluated in chronic HCV infection (El-Bassiouny et al., 2008). The aim of this work was to assess the possible role of sFas and sFasL in the response of patients with HCV infection to treatment with pegylated interferon (PEG- IFN) and Ribavirin. Also, we tried to use sFas and sFasL as predictive markers for the combination therapy response in patients with chronic HCV.

Materials and methods

Patients

This study is a prospective study consists of 50 naïve patients to be treated with PEG-IFN-α2a plus ribavirin for 48 weeks. The inclusion criteria were set to avoid the confounding variables effect and to diminish the selection bias. The inclusion criteria were as follows: adult (18–60 years old) with established chronic HCV infection, liver enzymes elevation, positive HCV-RNA by PCR, and naive patients (not treated previously with any antiviral therapy). While the exclusion criteria were: decompensated hepatic disease, evidence of liver cirrhosis confirmed by histopathology, bilharzial infection, other causes of chronic hepatitis (e.g. Hepatitis B virus infection, drug-induced and autoimmune) alcohol intake, pregnant or nursing female and presence of any chronic systemic disease.

Procedure:

The demographic data, and other clinical features were reviewed and collected from the patients. Serum levels of sFas and sFasL were measured at baseline and at the end of treatment.

Treatment regimen:

All patients included in the study were treated with pegylated interferon (PEG-IFN-α2a) at a dose 180µg/week combined with ribavirin (1,000 mg/ day if the patients body weight were <75 kg and 1,200 mg/ day if the patients body weight were ≥75 kg) for 48 weeks. Relapse was assessed between the week 48 and the week 72. End of treatment response (ETR) was defined as undetectable HCV RNA at the end of treatment, while sustained virological response (SVR) was established by undetectable HCV RNA at 6 months after the end of treatment. Patients were categorized as having ETR (Responders), no ETR (Non responders), relapse or SVR.

Laboratory investigations:

Liver enzymes, serum albumin, serum bilirubin, serum creatinine, serum alkaline phosphatase, random blood sugar, concentrations were assayed using Beckman CX4 chemistry analyzer (NY, USA). Determination of Alpha-Feto

protein (AFP) and TSH were measured in all subjects. AFP was done using Axyam (USA). Ultrasonography and liver biopsy were performed for histological examination. Specimens of at least 2.5 cm in length, including a minimum of 12 portal tracts were considered reliable for grading and staging by modified Knodell's score (Ishak et al., 1995). This included a necro-inflammatory grading score (ranging from 0 = no activity to 18 = severe activity) and a fibrosis staging score (ranging from 0 = no activity to 6 = cirrhosis).

Investigated Parameters:

Quantitative determination of serum sFas, sFas-L were measured by using Enzyme-Linked ImmunoSorbent Assay (ELISA) technique according to manufacture instructions (IBL, Germany). HCV RNA was extracted from patients' sera via QlAmp Viral RNA mini extraction kit (QIAGEN, Germany). Quantitative HCV RNA was performed on Stratagene Mx3000P Real-Time PCR System (Agilent Technologies, California, USA) using the Taqman principle.

Statistical Analysis

Statistical package (SPSS, version 10.0) was used for data management. Descriptive statistics were presented as mean ± standard deviations for continuous variables, frequency and percentage for categorical variables. Student t-test (two sided) was used to test the significance of the difference between the mean value of the studied groups and chi-square test was used for comparison of categorical variables. The diagnostic value of each marker was assessed using Sensitivity, specificity, positive (PPV) and negative (NPV) predictive values. Receiver operating characteristic curves (ROC) were constructed to assess the validity of the markers in predicting the response by calculating the area under the curve (AUC). The Pearson correlation test was used to identify the correlation between sFas, sFasL and different clinic-pathological variables. The significance level was set at $p<0.05$.

Descriptive results:

Table (1) represents the descriptive data for the HCV- infected patients. The mean age was 40.1 ± 7.9 years and 68% (34) of them were males. Pre-treatment viral load was 2.3 X 10^9 ± 1.1 x 10^9 IU/ml, the mean necroinflammatory activity was 5.9 ± 1.2 and the mean fibrosis stage was 3.1 ± 0.8 by Ishak score in histopathological findings of liver Biopsies. Thirty two (64%) patients were responders while 18 (36%) patients were nonresponders.

sFas and sFasL levels according to the pattern of response:

No difference was found in sFas levels between responders and non-responders at baseline level (117.7 ± 37.9, 118.6 ± 32.4 pg/ml, respectively), table 1. While the baseline sFasL level was significantly higher in responders (p= 0.000), figure 1. After treatment, both sFas and sFasL levels were significantly higher in responders (p=0.015, 0.000, respectively), figures 2&3.

Behavior of sFas and sFasL after treatment:

After treatment, both sFas and sFasL levels increased in responders as compared to their baseline levels (table 1, figure 4). While their levels decreased in non-responders (table 1).

Correlations with sFas and sFasL levels:

Apart from the significant positive correlation between sFasL and ALT, (r = 0.28, p = 0.046) (figure 5), no statistically significant correlations were found between sFas or sFasL and liver enzymes, viral load, necroinflammatory changes and extent of fibrosis in liver tissue specimens as shown in tables 2,3.

Baseline sFasL as a marker for response:

Receiver operating characteristic (ROC) curve analysis of sFasL as a marker for ETR to therapy (table 4) showed that at a cutoff of 156.5 pg/ ml, the area under the curve (AUC) was 86% with sensitivity 87.5%, specificity 78.8%, PPV 88.9% and NPV 81.8%. ROC curve analysis of sFasL as a marker for SVR (table 5, figure 6) showed that the same cutoff (156.5 pg/ ml) gives AUC 85.4%, sensitivity 86.2%, specificity 78.7%, PPV 87.8% and NPV 81.8%.

Table (1): Demographic and laboratory Findings of all studied patients.

	Responders ***n*=32 (64%)**	**Non responders** ***n* =18 (36%)**	**All patients** ***n* =50 (100%)**	***p*-value**
Age at treatment (Years)	41.3 ± 8.6	37.9 ± 6	40.1 ±7.9	0.14
Male gender, n (%)	21 (65.6)	13 (72.2)	34 (68)	0.439
BMI	28.2 ± 9.7	24.6 ± 8.6	26.9 ± 9.4	0.19
ALT (IU/ml)	62.5 ± 21.3	53.2 ± 14.6	59.1± 19.5	0.1
AST (IU/ml)	65.8 ± 25.5	56.8 ± 21	62.6 ± 24.2	0.2
AFP (ng/ml)	15.0 ± 15.4	9.5 ± 4.7	13.0 ± 12.8	0.15
TSH (mIU/ml)	3.7 ± 0.6	3.5 ± 0.9	3.6 ± 0.7	0.45
Viral load $x10^9$ (IU/ml)	2.6 ± 1.3	1.7 ± 0.7	2.3 ± 1.1	0.79
Ishak score:[a]				
(Grade) activity	5.8 ± 1.1	6 ± 1.3	5.9 ± 1.2	0.6
(Stage) fibrosis	3.1 ± 0.9	3.1 ± 0.6	3.1 ± 0.8	0.76
Relapse, n (%)	3 (9.4)	-	3 (6)	
SVR, n (%)	29 (90.6)	-	29 (58)	
sFas before treatment (pg/ml)	117.7 ± 37.9	118.6 ± 32.4	118 ± 35.7	0.936
sFas after treatment (pg/ml)	121.8 ± 40	97.4 ± 10.7	113 ± 34.5	0.015*
sFasL before treatment (pg/ml)	200.9 ± 28.7	146.9 ± 31.0	181.5 ± 39.2	0.000*
sFasL after treatment (pg/ml)	229.6 ± 29.0	131.4 ± 28.5	194.3 ± 55.5	0.000*

Data are shown as mean ± SD

BMI, Body mass index; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; AFP, Alphafetoprotein; TSH, Thyroid stimulating hormone; SVR, sustained virological response; [a] **Ishak et al., 1995**; *significant.

Table (2): Correlations between sFas and some laboratory and histopathological parameters among HCV patients

sFas / Variables	*r*	***p*-value**
AST	0.084	0.56
ALT	-0.118	0.41
Viral load	-0.58	0.68
sFasL	-0.1	0.467
Fibrosis (F)	0.089	0.54
Necroinflammatory activity (A)	0.04	0.765

Table (3): Correlations between sFasL and some laboratory and histopathological parameters among HCV patients

sFasL / Variables	*r*	***p*-value**
AST	0.15	0.29
ALT	0.28	0.046*
Viral load	-0.022	0.879
sFas	-0.1	0.467
Fibrosis (F)	0.048	0.74
Necroinflammatory activity (A)	0.068	0.637

*significant

Table (4): sFasL as a marker for ETR to combination therapy

Test	Cut off	Sensitivity %	Specificity %	PPV %	NPV %	p-value	AUC %
sFasL pg/ml	156.5	87.5	78.8	88.9	81.8	0.000	86.0

Table (5): sFasL as a marker for SVR to combination therapy

Test	Cut off	Sensitivity %	Specificity %	PPV %	NPV %	p-value	AUC %
sFasL pg/ml	156.5	86.2	78.7	87.8	81.8	0.000	85.4

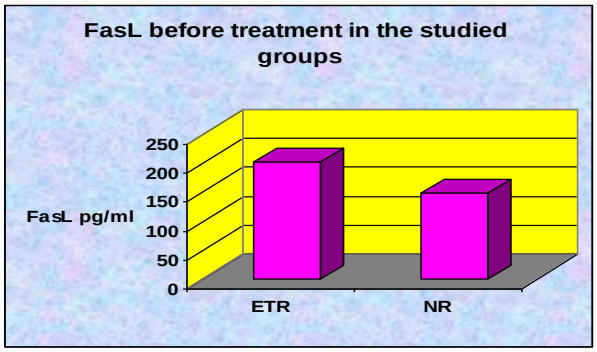

Figure (1) *p-value=0.000.

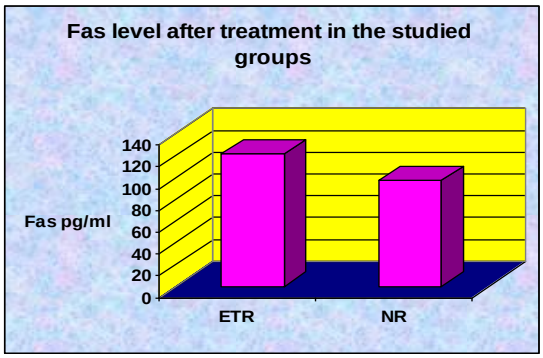

Figure (2) *p-value=0.015.

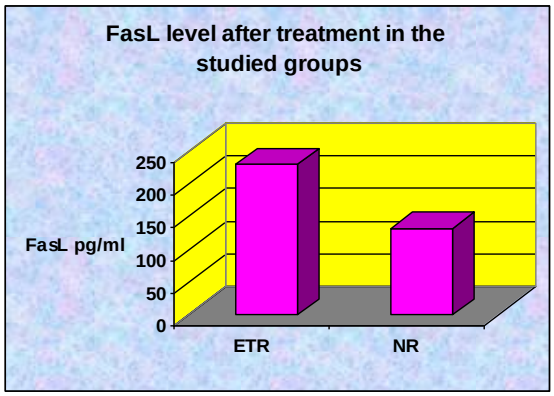

Figure (3) *p-value =0.000.

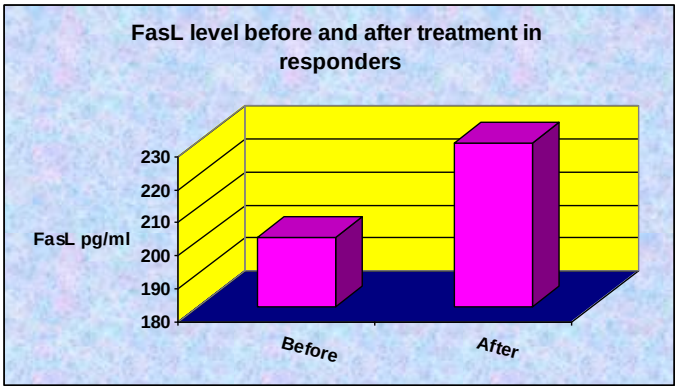

Figure (4) *p-value=0.0002.

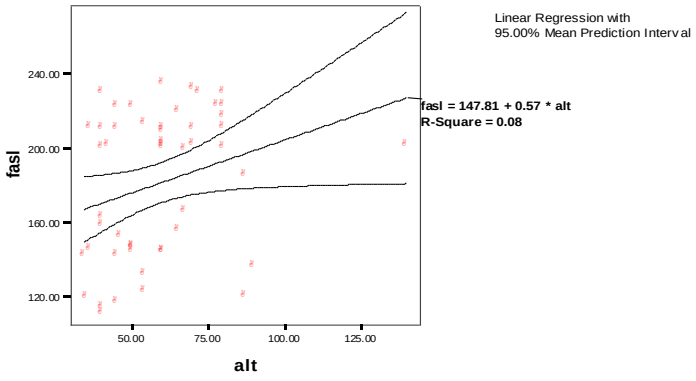

Figure (5): Correlations between serum sFasL and ALT among chronic HCV patients

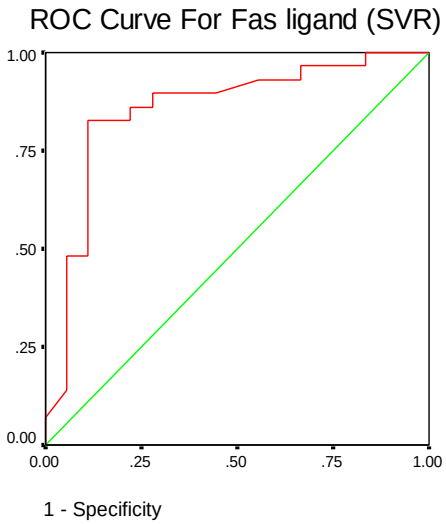

Figure (6): ROC curve for sFasL as a predictor of SVR

Discussion

Hepatitis C virus (HCV) infection and its related hepatic disease is considered to be one of the most common causes of chronic liver diseases worldwide (Seeff, 2002). Its prevalence varies through the world, with the highest prevalence reported in Egypt (Breban et al., 2013). About 80% of HCV patients are prone to develop chronic hepatitis (Boyer and Marcellin, 2000). Therefore, studying the immunological status of HCV patients and identifying which patients have a chance of a better response to therapy, may be helpful in the management of HCV patients more successfully (Davis et al., 2003).

Apoptosis has been implicated to have a significant role in the pathogenesis of hepatitis C-induced hepatic damage (Fischer, et al., 2007). Although the significance of studying of the cellular events related to the apoptotic signaling is well established, practically serum markers may provide non- invasive methods that could be helpful in evaluating HCV patients, particularly during the antiviral therapy (Shiavon, et al., 2011). In the present study, we have evaluated serum levels of sFas and sFasL according to the pattern of response, sFas basal level was not significantly different among responders and non-responders, but increased after treatment in responders and this goes in agreement with Zekri, et al., 2007, who found that sFas levels were significantly increased in the patients who achieved SVR after PEG-IFN Plus ribavirin combination therapy. This elevation of sFas levels which was observed during PEC-IFN plus ribavirin therapy could be actually reflects a stimulation of the hepatic fas expression, contributing to the IFN-induced HCV clearance process (Shiavon, et al., 2011). In our study, the baseline sFasL levels were significantly higher among responders as compared to non-responders. In contrast, Toyoda et al., 2000 reported no significant differences in baseline levels of sFasL among patients with SVR and non-responders. However, this discrepancy might be explained by the treatment with the conventional IFN monotherapy in the Japanese study. In the same context, Zekri, et al., 2007 found no significant differences in sFasL baseline levels regarding the response to PEG-IFN plus ribavirin combination therapy. However, this might be due to the small number of patients included in their study, which could limit the results interpretation (Shiavon et al., 2011).

In the present study, a significant increase in sFasL levels was observed after treatment in responders (p=0.0002). In harmony to our results, Zekri et al., 2007 and Shiavon et al., 2011 reported a significant elevation of sFasL level during HCV treatment, especially in those who achieved SVR.

The previous results suggest that sFasL may have a role in the cytokine-induced HCV clearance process. The high concentrations of sFasL that were observed to be persistent after the end of HCV treatment among the responders may be an indication of a residual effect of PEG-IFN or reflects a stronger sFasL induction in those patients who seems to be more prone to the virological response (Schiavon, et al., 2011).

In the current study, pretreatment high sFasL was the only predictor for response to combination therapy, ROC curve analysis of sFasL as a marker of response to combination therapy showed that, a cutoff 156.5 pg/ ml gives excellent sensitivity, specificity, PPV, NPV for both ETR and SVR with AUC of 86.0% and 85.4%, respectively. These results goes in agreement with Zekri, et al., 2007 who concluded that sFasL have the potential to be used as a serological marker for response to PEG- IFN therapy.

Shiavon et al., 2011 found similar results, but the absence of an established cutoff value may limit the interpretation of their results. However, the behavior of the sFas and sFasL during HCV treatment was the most important result in their study, and the absence of a predictive cutoff value did not affect this result.

Conclusion

In conclusion, the virological response of HCV patients to PEG-IFN plus ribavirin combination therapy was associated with an elevation of the serum concentrations of sFas and sFasL in responders suggesting that apoptosis may have a key role in HCV clearance induced by treatment with PEG-IFN plus ribavirin combination therapy. Pretreatment sFasL may be one of the host related factors used for evaluating the response to therapy and have the potential to be used as a serological marker for response to PEG-IFN and ribavirin therapy.

Acknowledgement

We thank Dr. Ola Nada, Pathology Department of Faculty of Medicine, Ain Shams University, Cairo, Egypt, for generous and sincere help in this study.

References:

- Boyer N, Marcellin B (2000). pathogenesis, diagnosis and management of hepatitis C virus. J Hepatol, 32: 98–112.
- Breban, R., Doss, W., Esmat, G., Elsayed, M., Hellard, M., Ayscue, P., Albert, M., Fontanet, A. and Mohamed, M. K. (2013). Towards realistic estimates of HCV incidence in Egypt. Journal of Viral Hepatitis, 20: 294–296.
- Cascino I, Fiucci G, Papoff G, Ruberti G (1995). Three functional soluble forms of the human apoptosis-inducing Fas molecule are produced by alternative splicing. *J Immunol*, **154:** 2706-2713.
- Cornberg, M., Razavi, H. A., Alberti, A., Bernasconi, E., Buti, M., Cooper, C., et al (2011). A systematic review of hepatitis C virus epidemiology in Europe, Canada and Israel. *Liver International*, *31*(s2), 30-60.
- Darwish MM, Hafez SF, Mohammed MA, Elaish NM and El Zayadi AM (2012).The status of Fas and Fas Ligand and expression on peripheral mononuclear cells in chronic hepatitis C virus infection and hepatocellular carcinoma. Egyptian Journal of Biomedical Sciences.8:17-46.

- Davis, G. L., Albright, J. E., Cook, S. F., & Rosenberg, D. M. (2003). Projecting future complications of chronic hepatitis C in the United States. *Liver Transplantation*, *9*(4), 331-338.

- El Bassiouny A, El-Bassiouni N, Nosseir M, Zoheiry M, El-Ahwany E,Salah F, Omran Z, and Ibrahim R (2008).Circulating and hepatic Fas expression in HCV-induced chronic liver disease and hepatocellular carcinoma. Medscape J Med. 10(6): 130.

- Fischer R, Baumert T, Blum H (2007). Hepatitis C virus infection and apoptosis. World J Gastroenterol.13(36):4865-72.

- Gruss HJ (1996). Molecular, structural, and biological characteristics of the tumor necrosis factor ligand superfamily. *Int J Clin Lab Res*, **26:** 143-159.

- Guicciardi ME and Gores GJ (2005). Apoptosis: A mechanism of acute andchronic liver injury. Gut.54(7):1024-33.

- Guicciardi ME, Malhi H and Gores GJ (2010). Hepatocyte death: a clear and present danger.Physiol Rev.90(3):1165-94.

- Hayashi, N., & Mita, E. (1999). Involvement of Fas system-mediated apoptosis in pathogenesis of viral hepatitis. *Journal of viral hepatitis*, *6*(5), 357-365.

- Iio, S., Hayashi, N., Mita, E., Ueda, K., Mochizuki, K., Hiramatsu, N., et al. (1998). Serum levels of soluble Fas antigen in chronic hepatitis C patients. *Journal of hepatology*, *29*(4), 517-523.

- Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H,Desmet V,Korb G, MacSween R , Phillips M , Portmann B, Poulsen H , Scheuer P, Schmid M, Thaler H (1995). Histological grading and staging of chronic hepatitis.J Hepatol.22(6):696-9.

- Lavanchy D (2009). The global burden of hepatitis C. *Liver Int*, **29:** 74-81.

- Martín, J., Quiroga, J. A., Navas, S., Pardo, M., & Carreño, V. (1999). Modulation by biologic response modifiers of hepatitis C virus antigen-independent cytokine secretion in blood mononuclear cells. *Cytokine*, *11*(4), 267-273.

- Okazaki, M., Hino, K., Fujii, K., Kobayashi, N., & Okita, K. (1996). Hepatic Fas antigen expression before and after interferon therapy in patients with chronic hepatitis C. *Digestive diseases and sciences*, *41*(12), 2453-2458.

- Papoff, G., Cascino, I., Eramo, A., Starace, G., Lynch, D. H., & Ruberti, G. (1996). An N-terminal domain shared by Fas/Apo-1 (CD95) soluble variants prevents cell death in vitro. *The Journal of Immunology*, *156*(12), 4622-4630.

- Schiavon LL, Narciso-Schiavon JL, Carvalho-Filho RJ, Sampaio JP, El Batah PN, Silva GA, Carvente CT, Silva AE, Ferraz ML (2011). Evidence of a significant role for Fas-mediated apoptosis in HCV clearance during pegylated interferon plus ribavirin combination therapy. Antiviral Thera .16(3):291-298.

- Seeff LB (2002). Natural history of chronic hepatitis C. Hepatology, 36: S35.

- Toyoda M, Kakizaki S, Horiguchi N, Sato K, Takayama H, Takagi H, Nagamine T, Mori M (2000). Role of serum soluble Fas/soluble Fas ligand and TNF-alpha on response to interferon-alpha therapy in chronic hepatitis C. Liver.20(4):305-11.

- Zekri AR, Haleem HA, Esmat GE, Bahnassy AA, El-Din HM, Hafez MM, Sharaby AF, Sharaf H, Zakaria MS (2007). Immunomodulators, sFas and Fas-L as potential noninvasive predictors of IFN treatment in patients with HCV genotype-4.J Viral Hepat.14(7):468-77.