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THE CYTOKINE IP-10 IN CHRONIC HBV AND HCV INFECTION

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ABSTRACT

Introduction: IP-10 it has been studied as a predictor of treatment response in chronic HCV infected patients. The data for the HBV infection are not enough.

Aim: To compare IP-10 levels in patients with chronic HBV /CHB/ and HCV infection /CHC/ and their relation to liver disease and treatment response.

Material and methods: 20 patients - with CHC genotype 1 infection /on standard bi-therapy/ and 32 patients with CHB /21 pts - NUC; 11 pts - IFN/.

Results: The IP-10 did not correlate with sex, age, ALT and liver fibrosis. The basal IP-10 were lower in patients with CHB (p=0,017). There was a difference in IP-10 baseline levels among the HCV patients with or without RVR (p=0,007). A negative correlation was found between basal IP-10 and RVR (r= -0,508; p=0,008).

Conclusion: IP-10 could predict virological response in patients with CHC on standard bi-therapy, but not in HBV infected patients on standard therapy.

Key words: IP-10, chronic HCV, HBV infection, response.

INTRODUCTION

Globally, 350 million people are infected with chronic HBV[1] and up to 180 million people worldwide are infected with chronic HCV. The chronic viral infections are a major cause of chronic liver disease, cirrhosis and hepatocellular carcinoma [2, 3]. Despite increasing the number of available anti-viral agents, the treatment of chronic HBV and HCV infection is still a problem [4].

Numerous cytokines are involved in cell-mediated and humoral immune responses, as well as antiviral activity, viral clearance, apoptosis, and fibrogenesis [5].

IFNs are cytokines who signal to adjacent cells in the infected tissue, informing of a pathogenic state [6]. The IFN enhance the first defense against viral infections and modulate both innate and adaptive immune cells [7].

The hepatitis C virus interferes with the immune response and a balance between the innate and the adaptive response is pivotal in not only progression of the disease, but also eradication of HCV [8]. The administered IFN alfa used in the current standard therapy for HCV infection mimics the antiviral effects of endogenously produced IFN [9].

HBV is a non-cytopathic virus. Chronic infection with HBV is the result of an ineffective anti-viral immune response towards the virus [10, 11]. The exact mechanism by which HBV escapes immunity is still not known.

Elevated plasma levels of the ISG interferon-г-inducible protein 10 kDa (IP-10 or CXCL10) is common in HCV infection. IP-10 is a chemokine produced by endothelial cells, activated T cells (promoting a TH1 response) and hepatocytes during HCV infection, exerting its effects mainly through a G protein-coupled receptor CXCR3 [12]. IP-10 has been studied in the last years as a predictor of viral responsece in the treatment of HCV infection.

The data for the HBV infection are not enough.

AIM:

To compare IP-10 levels in patients with chronic HBV and HCV infection and their relation to viral replication, liver disease and treatment response.

PATIENTS AND METHODS

Patients. 52 patients with chronic viral infection were studied: 20 patients with CHC; 32 patients with CHB. All HCV patients were adults, had compensated liver disease, were seronegative for hepatitis B surface antigen (HBsAg) and for antibodies to HIV, and had the following characteristics: a positive test for anti-HCV antibody, an HCV-RNA level exceeding 1000 IU/mL, and 2 serum alanine aminotransferase values above the upper limit of normal within 6 months of treatment initiation. The HCV patients, had an HCV genotype 1 infection.

All patients with chronic HBV infection were adults, has apositive test for HBsAg, an HBV DNA detectalbe, and 2 serum alanine aminotransferase values above the upper limit of normal within 6 months of treatment initiation. All of these 52 patients had baseline and on third month of treatment serume samples available for analysis of IP-10 (baseline characteristics of patients shown in Table 1 and 2). For the patients with chronic HCV infection serum samples on first month of treatment were available too.

Table 1. Baseline Caracteristics of the HBV and HCV genotype 1patients

	HBV pts	HCV pts
Number of pts	32	20
Age /mean/	42	39
Male	25	10
Female	7	10
HBeAg /-/negative	21	-
F4 /METAVIR/	5	1

TREATMENT.

Treatment with PEG-IFN and Ribavirin was initiated in all patients with chronic HCV infection.

In 21 of patients with chronic HBV infection a treatment with NUC were initiated and in 11 patients – a PEG-IFN treatment (According to the guidelines for the treatment and management of chronic viral hepatitis). (11 pts - Entecavir, 8 - Tenofovir, 2 - Telbivudine). 21 patients were HBeAg negative, 11 - HbeAg positive before the therapy (2 of them on Peg-IFN alfa 2a therapy).

HCV-RNA and HBV-DNA Quantification. The concentration of HCV-RNA and HBV-DNA was determined by standart real-time PCR method.

HCV-RNA quantification was performed on days 0, on first month , third month at the end of treatment, and 24 weeks after the completion of treatment /in the patients who were reached this stage of treatment course in time of presenting this article/.

HBV-DNA quantification was performed on days 0 and on every 3 months during the treatment.

Classification of Viral Kinetic Response. Patients were classified as achieving RVR if the HCVRNA levels during the first 4 weeks of therapy were indetectales.

Classification of Treatment Outcome. Patients were classified as achieving SVR if serum HCV-RNA/HBV-DNA was undetectable 24 weeks after completion of therapy /for INF treated patients/.

Genotyping. Genotyping of HCV was performed using INNO-LiPA HCV II (Innogenetics NV, Ghent, Belgium).

IP-10 Quantification. Quantification of human IP-10 was performed using Ray Bio ® Human IP-10 ELISA kit, on serum samples. All samples were stored at -70°C until assayed.

Liver Biopsies.

Liver biopsies were perfomed in 17 patients with chronic HCV infection and in 26 of patients with chronic HBV infection. 4 of HBV patients without biopsy had a proved liver cirrhosis (F4).

5 HBV patients were with liver cirrhosis, and the rest had a fibrosis stage F 1-3 (Metavir). One of HCV patient had a compensated liver cirrhosis, and the rest had a fibrosis stage F 1-3 (Metavir).

Statistical Methods.

Standard statistical analyses were performed using SPSS® v. 17.0.

All reported *P* values are 2-tailed, and *P* values less than .05 were considered significant.

Informed Consent and Ethical Committees.

Written informed consent was obtained from each participating patient. Ethical committees at each study center approved the treatment study.

RESULTS

Quantification of IP-10 was performed in all 52 patients. The baseline levels of IP-10 were significantly lower in patients with HBV infection (mean 76±67,4 pg/ml) than in HCV patients genotype 1 (124,7±73,7) (P=0.017) .

The levels of IP-10 did not correlate with sex, age, ALT, histology activity and liver fibrosis in both (HBV and HCV) groups of patients (Table 2.).

Table 2. IP-10 basal leves and gender, age, ALT, histology activity and liver fibrosis

characterics	**HBV pts**	**P**	**HCV pts**	**P**
Age	42±11,5	0.446	39±11,7	0.795
Gender		0.767		0.290
Male	25		10	
Female	7		10	
Fibrosis		0.815		0.524
1	10 pts		11 pts	
2	9		4	
3	6		1	
4	5		1	

Histological activity		0.264		0.660
0			2	
1	11		11	
2	8		3	
3	7		1	
ALT	68±92	0.455	68±38	0.079

Chronic HCV infection.

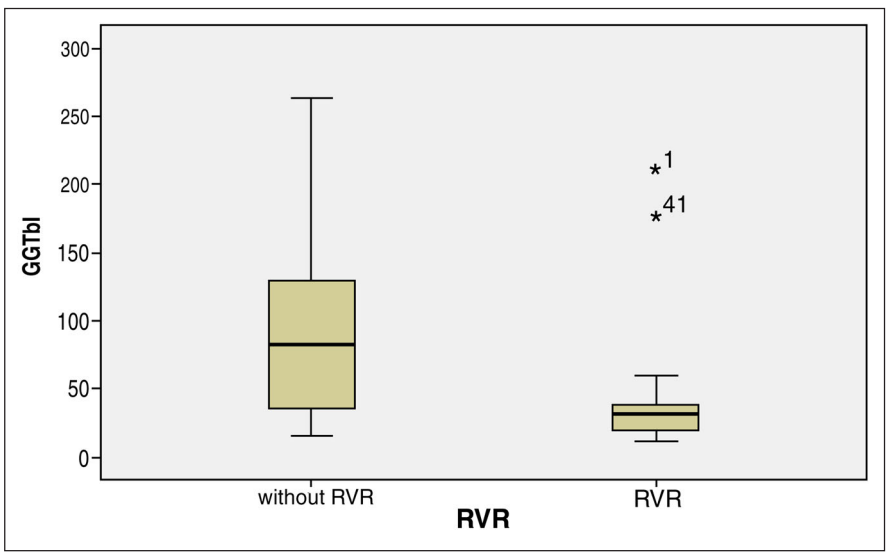
In 14 of 20 HCV patients the basal IP-10 levels were under 150 pg/ml, and in the rest 6 patients the levels were between 150 and 400 pg/ml. The HCVRNA baseline levels were under 400 000 IU/ml in 13 patients and above 400 000 IU/ml in 7 patients.

In 10/14 patients with basal IP-10 levels under 150 pg/ml, the HCVRNA basal levels were under 400 000 IU/ml. In 3/6 pts with IP-10 levels above 150 pg/ml, HCVRNA basal levels were above 400 000 IU. But no statistically correlation was found.

We found a correlation between basal IP-10 and GGT in HCV patients (r=0,44; p=0,007). In patients without achieved RVR the baseline levels of GGT were higher /89 U/l/ than in these with RVR /33 U/l/, but not significant difference was found /P=0.075/. Our results on larger group of patients /55 pts/ showed a statistical significant difference between GGT basal in patients who achieved RVR and who did not achieved RVR /P=0.001/ (Figure 1.).

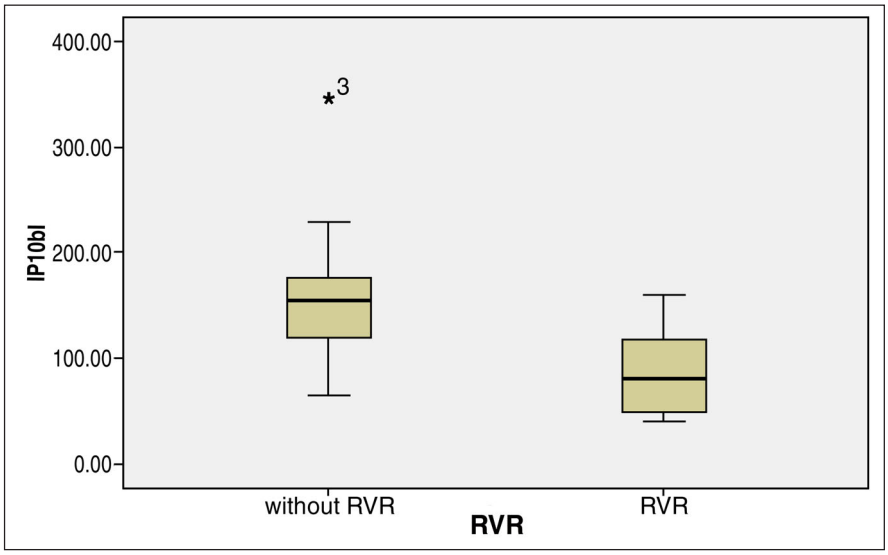
Also a correlation was found between basal GGT and the respose to treatment in this group of 55 pts /with or without RVR/ P=0.001, r =-0.371/.

Figure 1. Difference in baseline levels of GGT between patients /55 pts group/ with achieved RVR and without RVR



There was a significant difference in IP-10 baseline levels among the HCV patients with or without RVR (p=0,007) . Higher levels were found in patients who did not achieve a RVR (Figure 2.).

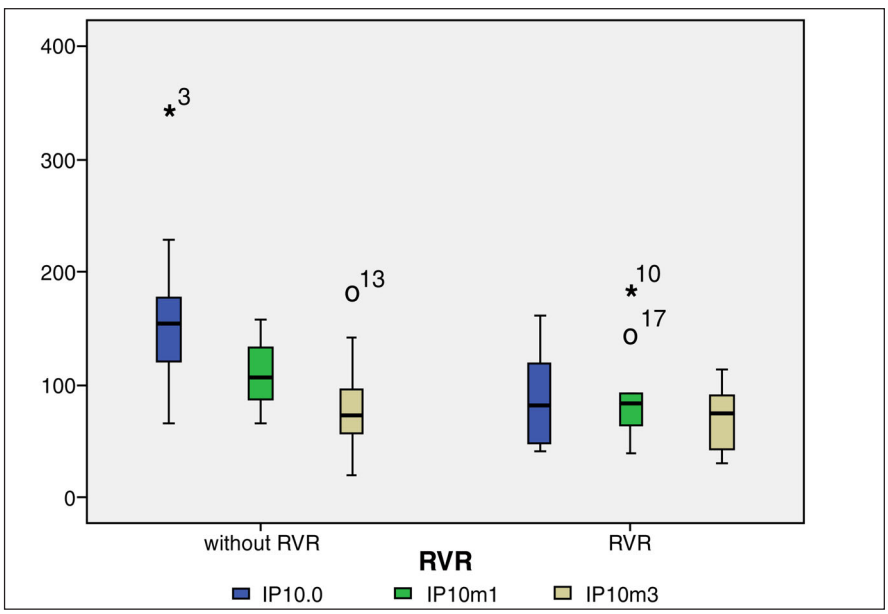
Figure 2. IP-10 basal levels in HCV infected patients – achieved or did not achieved RVR.



There was a negative correlation between basal IP-10 and RVR (r= -0,508; p=0,008).

During the treatment IP-10 levels dropped progressively more than 50% on the 3rd month compared to baseline in patients who did not achieved RVR, but with EVR /p=0.005/ (Figure 3).

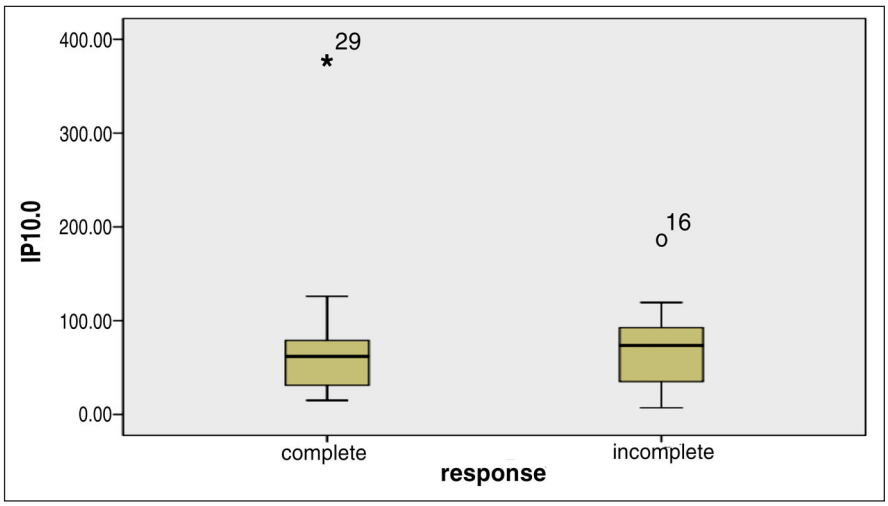
Figure 3. IP-10 levels during standard bi-therapy in pts with chronic HCV infection and viral response



Chronic HBV infection.

In HBV infected patients the levels of IP-10 were not related to viral response. We separated the 32 HBV pts according to the response on the third month of therapy – patients with detectable HBV-DNA on third month (for simplicity named "incomplete response") and patients with undetectable HBV-DNA ("complete response"). There was no statistical difference in the basal levels of IP-10 between the both group /P =0.613/ (Figure 4).

Figure 4. IP-10 basal levels and treatment response in HBV patients



There was no a correlation too /P=0.603/. There was no significant difference between IP-10 baseline and on the third month in the HBV patients group /P=0.640/ .

No statistical difference was found between IP-10 basal and on 3rd month in group of IFN /P=1.00/ and in group of NUC /P=0.455/, but a a similar decrease in IP-10 levels of about 33% in the course of therapy / basal and third month/ is established in the both groups (Table 3.)

Table 3. IP-10 levels during standart therapy in HBV infected patiens - IFN or NUC

Therapy	IP-10 pg/ml basal - median	IP-10 3rd month - median
NUC	59,8	42
IFN	73,6	58,5

DISCUSSION:

It was reported that pretreatment IP-10 is a predictor factor of RVR and respectively of SVR following interferon and ribavirin therapy in HCV-infected patients. But no enough data about the Bulgarian population of HCV infected patients. No enough data also about the role of IP-10 in chronic HBV infection and treatment response.

In this study, we focused on the potential predictive value of pretreatment IP-10 levels in serum in patients chronically infected with HCV of genotype 1 and HBV patients /NUC and IFN therapy/. We aimed also to compare the IP-10 levels in HCV and HBV infected patients.

Initially we examined the utility of baseline analysis of IP-10 in predicting RVR in all HCV genotype 1 patients, and found that baseline IP-10 levels were significantly associated with initial viral kinetic response. Higher levels were found in patients who didn't achieve a RVR. We found a negative correlation between GGT and response to standard bi-therapy in chronic HCV infection. Usually elevated GGT levels are associated with liver steatosis and a mononuclear infiltration.

Next, we evaluated potentially useful cutoff levels of baseline IP-10 and compared the utility of these cutoff levels with more established viral markers of response. 9 out of 10 patients with detected RVR had baseline levels of IP-10 below 150 pg/ml. However, it should be stressed that once therapy /in HCV pts/ has been started, decisions regarding possible discontinuation should be based on insufficient virological response or intolerance resulting from side effects, and not on the baseline IP-10 level.

The IP-10 concentration mirrors the degree of local chemokine signaling in HCV-infected hepatocytes aimed at recruiting mononuclear cells to the liver to combat the ongoing viral infection. It is hypothesized that as the intrahepatic viral replication diminishes during treatment, so does the chemokine signaling from infected hepatocytes induced by the viral replication. If the virus is not eradicated after completion of therapy, intrahepatic viral replication, and thus chemokine signaling, resumes once the antiviral therapy is terminated /M. Lagging et al. Hepatology, December 2006/.

The baseline levels of IP-10 were significantly lower in patients with HBV infection than in HCV patients' genotype 1. According to our results the IP-10 levels not correlated with viral load in HBV and HCV patients. IP-10 baseline serum levels could not predict virological response in HBV infected patients on treatment with nucleotide analogs or IFN.

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