Role of serum interlukine 29 and interferon γ inducible protein 10 as predictive markers for hepatitis C treatment response

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Abstract: Background: HCV is a global health problem and the World Health Organization estimates that at least 170 million people representing approximately 2% of the world's population are infected with HCV worldwide, with most of these concentrated in developing countries. In Egypt there is high prevalence of hcv (~14.7 %), with high proportion of patients with genotype 4 have a low response rate to antiviral therapy Until recently, the standard of care for CHC was lengthy dual therapy with pegylated interferon plus ribavirin with modest success rates ~50 % of patients, severe adverse events and variation in treatment response between genotypes. IL29 is a small-molecular weight protein (22 kDa) helical structure which was functionally described as an 'inflammatory' chemokine IL 29 can be induced by hepatocytes and other cell types within the liver, including Kupffer cells, stellate cells and dentrtic cells that may be considered as the main producer (Bolen et al., 2014). IL-29 inhibits the replication of HCV IP10 is a small-molecular weight protein (10 kDa) which was functionally described as an 'inflammatory' chemokine IP-10 plays an important role in the pathogenesis of HCV infection. In HCV infection, the inflammatory cells that infiltrate the liver are mainly antigen-nonspecific T-helper 1 (Th1) lymphocytes. **Aim:** This study aimed to the aim of this work is to assess the value of pretreatment measurement of serum level of interleukin 29 and serum interferon gamma inducible protein 10 (ip-10) on the response of treatment of interferon therapy in patients suffering from CHC. **Patients and Methods:** 60 patients with CHC subdivided into 2 groups according to response to interferon therapy and 30 healthy controls. The serum IL-29 and IP-10 levels were measured by enzyme-linked immunosorbent assay (ELISA). Pretreatment viral load was done in CHC patients in addition to viral load after 12 weeks of treatment was done for responders only, serum AST, ALT, ALP, were measured in all population. **Results:** The results showed highly statistically significant difference regarding pretreatment viral load between responders and non responders, statistically significant difference among all studied groups regarding their serum (IL29), and serum IP-10they had negative no statistically significant correlation with each other. Serum IP-10 and serum IL-29 had negative no statistically significant correlation with each other. ROC curve analysis using a cut off value of serum IP-10 >0.6 ng/ml for accurate prediction non responders and cut off of for accurate prediction of responders for serum IP-10 levels was identified to be >1.41 ng/ml. while for serum IL-29 the best cut off fro prediction of responders >>1.4 ng/ml was a good pretreatment selection of responders to treatment and cut off level for IL-29 >=2.83 ng/ml for pretreatment selection of responders, while for pretreatment Viral load the best cut off value for discrimination between responders and non responders was >1400 IU/ml These cut –off value were found to be sensitive and specific for prediction of response treatment in patients with CHC. **Conclusion:** The results suggested that pretreatment measuremrnt the serum IP-10 and serum IL-29 can be used as predictors for response of treatment in patients with CHC.

[Soheir Abd El-Rahman Saleh, Sabah Ibraheem Abd-Rahem, Asmaa Mohamed Ibrahim. **Role of serum interlukine 29 and interferon γ inducible protein 10 as predictive markers for hepatitis C treatment response.** *Nat Sci* 2018;16(7):29-33]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 6. doi:10.7537/marsnsj160718.06.

Key words: HCV, CHC, IL-29, IP-10, Predictors for interferon therapy

1. Introduction:

HCV is a global health problem and the World Health Organization estimates that at least 170 million people representing approximately 2% of the world's population are infected with HCV worldwide, with most of these concentrated in developing countries (1). In Egypt there is high prevalence of hcv (~14.7 %), with high proportion of patients with genotype 4 have a low response rate to antiviral therapy (2).

Until recently, the standard of care for CHC was lengthy dual therapy with pegylated interferon plus ribavirin with modest success rates ~50 % of patients, severe adverse events and variation in treatment response between genotypes (3).

Later years have seen the forthcoming of the new direct acting antivirals (DAA), and all current treatment recommendations for CHC patients from the European Association for the Study of the Liver contain at least one DAA (4).

Development of viral resistance to DAAS and

also being quite expensive and not supported by National Health Insurance in every country (5).

Therefore peg-IFN/RBV treatment still has a role to play in treatment of patients with CHC, and the need for markers that can predict successful treatment outcomes to peg-INF-α/RBV are still needed, and great effort has been put into identifying biomarkers to predict rapid virological response (5).

Of the most promising chemokine biomarkers candidate are serum IL 29 (6), and serum (IP-10) (7). IL29 is a small-molecular weight protein (22 kDa) helical structure which was functionally described as an 'inflammatory' chemokine (8).

IL 29 can be induced by hepatocytes and other cell types within the liver, including Kupffer cells, stellate cells and dentrtic cells that may be considered as the main producer (9). IL-29 inhibits the replication of HCV (10).

IP10 is a small-molecular weight protein (10 kDa) which was functionally described as an 'inflammatory' chemokine (8).

IP10 is produced by hepatocytes and sinusoidal endothelial cells (11). The expression of IP10 in the liver can be induced directly by HCV itself, also, HCV proteins such as NS5A and core, alone or in combination with proinflammatory cytokines, can induce IP-10 gene expression and secretion in human hepatocyte derived cells (12).

IP-10 plays an important role in the pathogenesis of HCV infection. In HCV infection, the inflammatory cells that infiltrate the liver are mainly antigen-nonspecific T-helper 1 (Th1) lymphocytes. (13).

This immune reaction results in ongoing tissue damage and progressive liver disease (14).

The aim of this work is:

To assess the value of pretreatment measurement of serum level of interleukin 29 and serum interferon gamma inducible protein 10 (ip-10) on the response of treatment of interferon therapy in patients suffering from CHC.

2. Patients and methods

Study population:

This study is a prospective case – control study which will take place in the clinical pathology department faculty of medicine for girls Alazhar University. The study carried out on 60 patients suffering from CHC receiving treatment in form of pyglated interferon and ribavirin in addition to 30 apparently healthy individuals referred as a control group.

The studied patient's were divided into 3 groups:

Group I: included 20patients with CHC not responding for treatment (9 males and 11 females), their ages ranged between 21 – 69 years.

Group II: included 40 patients with CHC patients responding for treatment (24 males and 16 females), their ages ranged between 18 – 66 years.

Group III: Included 30 ages and sex matched apparently healthy volunteers. (15 male and 15 females), their ages ranged between 18 – 67 years.

Sampling:

For all subjects, the followings were done:

Bloodsample were collected from each individual under aseptic condition by clean veinupuncture without venous stasis. All studied cases were subjected to detailed history taking, thorough clinical examination, and laboratory investigation with emphasize on:

Lab Investigations:

A- Serum AST, ALT, ALP

B- Pretreatment viral load and after 12 weeks of treatment by PCR.

C- Serum IP10: It was detected by Elisa kits (Human interferon induced protein 10 (IP10) ELISA, Glory science Co., Ltd).

D- Serum interlukin 29 (IL29) by Elisa kits (Human interlukin 29 (IL29) ELISA, Glory science Co., Ltd).

ELISA for quantitation of serum Ip10:

Statistical analysis:

The SPSS statistical software package version20.0 (Armonk, NY: IBM Corp) was used for all of the statistical analyses.

Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level. P value non-significant difference if $P > 0.05$, significant difference if $P < 0.05$ and highly significant difference if $P < 0.001$.

3. Results:

The results of this study revealed that:

- Comparing the 3 studied groups there was statistically significant difference regarding their serum (IL29) (p value < 0.033).
- Comparing the 3 studied groups there was statistically significant difference regarding their serum (IP10) (p value <0.037).
- There was negative no statistically significant correlation between serum IP-10 and serum IL-29.
- Serum IP-10 had positive no statistically significant correlation with serum ALP, serum ALT and serum AST, while had a negative no statistically significant negative correlation with BMI, age of patients and pretreatment viral load (PCR1).
- Serum (IL29) had a positive statistically significant correlation with serum ALT, and a positive statistically no significant correlation with serum ALP and serum AST while had a negative statistically no

significant correlation with serum BMI, age of patients and pretreatment viral load (PCR1).

- ❖ For accurate prediction of failure of treatment a threshold for serum IP-10 levels was identified to be >0.6 ng/ml This IP-10 cut off value was able to identify NR to antiviral treatment at baseline.
- ❖ For accurate prediction of responders a threshold for serum IP-10 levels was identified to be >1.41 ng/ml by computing an ROC curve. This IP-10 cut off value was able to identify SVR to antiviral treatment at baseline
- ❖ Regarding serum IL-29for accurate prediction of failure of treatment a threshold for serum il-29 levels was identified to be >= 2.83 ng/ml by computing an ROC curve. This IL-29 cut off value was able to identify NR to antiviral treatment at baseline
- ❖ While serum IL-29 levels predicting responders (SVR) for treatment with area under the curve (AUC) was 0.657, pretreatment serum IP-10 at cutoff value of > 1.4 ng /ml can predict well responders (SVR) to treatment.

The results of this study were discussed and compared with other studies.

4. Discussion:

Viral hepatitis was estimated to be the 7th leading cause of mortality globally. About half of this mortality is attributed to hepatitis C virus (HCV), a primary cause for liver fibrosis, cirrhosis and cancer (15). Action to combat viral hepatitis has now been integrated into the United Nations' 2030 Agenda for Sustainable Development (15). 92–149 million people, representing approximately 2% of the world's population, are chronically infected with HCV (16).

One of the countries most affected by HCV is Egypt. The Egyptian Demographic and Health Surveys (EDHS) measured antibody prevalence among the adult population aged 15–59 years at 14.7% in 2009 and at 10.0%12 in 2015—substantially higher than global level (15).

Egyptian patients infected with HCV with high proportion of patients with genotype 4 which alone accounted for 94% of HCV infections having a low response rate to antiviral therapy, so searching for predictive factors in these groups of difficult-to-cure patients is needed (15).

CHC was classically treated with recombinant PEG-IFN alpha in combination with Ribavirin (RBV) with severe side effects occurring frequently, and still only approximately half of the patients being cured. Since 2014 HCV therapy improved drastically, as several direct acting antivirals targeting HCV NS3/4A protease, NS5A, or NS5B RNA-polymerase were approved (16).

Interferon gamma-inducible protein-10 (IP-10), also known as C-X-C motif ligand 10 (CXCL10), is an interferon (IFN)-γ /α and tumor necrosis factor alpha (TNF-α)-inducible chemokine that is highly expressed by a variety of cells, including hepatocytes, activated T lymphocytes, natural killer cells, and monocytes (17).

IP-10 plays an important role in the development and progression of liver disease, in which IP-10 binds to chemokine CXCR3 and recruits activated T lymphocytes to the liver parenchyma (17).

An elevated serum IP-10 level is associated with a poor response to antiviral therapy in Egyptian patients who were infected with HCV (presumably genotype 4) and who received a complete course of treatment consisting of IFN-α and ribavirin (2).

Furthermore, associations between serum IP-10 and HCV spontaneous clearance have shown the value of serum IP-10 for the early diagnosis of hepatic fibrosis and treatment outcomes with IFN-based therapy in patients with chronic hepatitis C (CHC) (18).

Interleukin (IL)-29, also known as interferon-lambda (IFN-λ), belongs to type III IFN family. It is classified among the class II cytokine receptor ligand family (19).

Like the Type-I interferons, IL 29 is induced by viral infections as well as by a range of mitogens, and uses STAT1 and STAT2 phosphorylation to mediate Type-I IFN-like functions such as upregulation of MHC class-I and induction of OAS-1 (20) CHC is associated with an expanded Tregs population, both in peripheral blood and liver (21).

Our study showed that there was no statistically significant difference among 3 studied groups regarding to their and gender (p value = 0.193, 0.495 respectively) and this meant good representation of both age and gender in our study among studied groups. Also according to the age our study showed that the mean age of NR (~ 46) years was higher than that of SVR and control group (~40) years for each with statistically significant difference of age between NR and both SVR and control group (p value = 0.033, 0.42 respectively) but there was no statistically significant difference between SVR and control group (p value =0.234).

This was in agree with **(22)** who stated that there was statistically significant difference between NR and SVR regarding to their age with in higher SVR in patients younger than 40 years and (23) who showed that there was statistically significant difference between responders and non-responders regarding to their age with more response matched with younger age.

In our study we found that no statistically difference between gender and response outcomes. as there was no statistically significant difference

between NR and SVR or control (p value =0.55 and 0.74 respectivly). This was in accordance with (22) who found no statistically significant difference in response to treatment regarding to gender.

The mean of body mass index (BMI) in our study was 31.47 kg/m^2 in NR and 27.1 kg/m in SVR, so there was highly statistically difference between low BMI and response to treatment (p value 0.001), This is also in agreeing with (24). who showed that obesity when defined as a BMI greater than 30 kg/m, is a negative predictor of response to hepatitis C treatment. Showed that Response was attained in 75% of non-obese patients (BMI <30), compared with only 50% of obese patients.

Our result serum showed that serum AST, ALT and ALP had highly statistically significant difference between patient groups (NR and SVR) and control group (p value <0.001) for each

We also found that there was no statically significant difference between NR and SVR as regarding their serum AST, ALT (p value =0.930and 0.666) respectively, This was in accordance with (5) et al 2016 who reported that the serum level of aminotrasferases, either normal or elevated, was not associated with SVR rate.

In our study there was highly statistically significant difference between NR and SVR as regard to the basal viral load this was in accordance with (5) who stated that the achievement of SVR was significantly higher in lower viral loads (<4x105 IU/ml, p <.001).

Our results suggested that an elevated serum IP-10 level is associated with a poor response to antiviral therapy in Egyptian patients who were infected with HCV (presumably genotype 4). IP 10 was higher in NR than SVR and control and there was statistically significant difference between responders and non-responders. In our study there was negative no statically significant correlation between serum IP 10 and serum IL29. this was in accordance with ***(25)*** who reported that there was no statically significant correlation between serum IP 10 and serum IL29.

Our results showed that regarding serum IL29, Its mean was higher in patient groups (SVR and NR) than in control group (vs), This agreed with results of ***(26)*** who reported that IL-29 serum level of HCV infected patient is elevated compared to those of healthy individuals, This different result might be attributed to the differences in the sample size which was relatively small (26 vs 90 patients).

Also there was statistically significant difference between NR and SVR being higher in SVR (p value = 0.028). But there was no statically significant difference between neither NR nor SVR with control group (p value=0.434, 0.303) respectively.

This was also in agreeing with ***(26)*** who reported that in CHC serum level of IL-29 was higher in patients who resolve HCV infection compared to non-responders patients.

We found also that there was no statically significant negative correlation of serum IL 29 and BMI this was not accordance with ***(23)*** who stated no statically difference between il29 and BMI But there was no statically significant negative correlation of serum il29 and, age of patients, basal viral load this was in accordance with ***(26)*** both reported the same as we reported.

In the present study the cut off level of pretreatment serum level of ip 10 to be used as predictive marker to differentiate the responders and non responders was >0.6ng/ml. These results is near to ***(27)*** who reported that the beat cutoff of IP-10 level of 3.59 ng/ml

In the present study the cut off level of pretreatment serum level of serum IL29 to be used as predictive marker to differentiate the responders and non responders was >=2.83 pg/ml. These results is near to (Claudio et al., 2013) who found that, IL-29 cut off level was 0.296 ng/ml.

Intended learning objectives: The learner will be able use the cut off values of serum IP-10, serum IL-29 to predict response of treatment in CHC patients.

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