ISSN: 2635 - 2826

Journal of Clinical & Biomedical Research

SCIENTIFIC Research and Community

Research Article

Open Access

Interleukin-28B (IL-28B) and Interferon –Gamma (INF-γ) Levels in Patients with Chronic Hepatitis C Viral (HCV) Infection

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ABSTRACT

Objectives: We aimed to studythe effect of Schsitosoma mansoni co-infection with hepatitis C virus (HCV) on IL-28B levels.

Design: We collected plasma from107outpatients(range30–81 years old) from six governates of Delta, Egypt attending Kasr Al-Aini Hospital, Cairo, Egyptin 2012–2014. Subjects were divided to three groups, 35 healthy controls, 50naïve chronic HCV patientsand 22S. mansoni/HCVco-infected patients.For all participants,anti-schistosomal antibodies levels, hepatitis B surface antigens (HBsAg), HCV viral loadand routine liver function tests were measured. We assayed IL-28B and IFN-γ plasma levels for all participants.

Results: We found that IL-28B levelsweresignificantly higher in S. mansoni/HCV co-infected patients than in HCV mono-infection. IFN-γ and IL-28B levels showed positive correlation in both infected groups. Patients with high HCV viral load had significantly higher IFN-γ and IL-28B levelswhether suffering from mono- or co-infection.

Conclusions: A strong link between IFN-γ and IL-28B in naïve chronic HCV patients whether mono- or co-infected with S. mansoni. This suggeststhat co-infection with S. mansoni might not affect IFN-γ levels, however, significantly increases IL-28B levels. Therefore, IL-28B plasma levels might be a useful novel biomarker forprognosis and therapy ofS. mansoni/HCV co-infection.

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Received: Aug 28, 2019; **Accepted:** Sep 06, 2019; **Published:** Sep 11, 2019

Keywords: Hepatitis C Virus, Schistosoma Mansoni, IFN-γ, IL-28B, Chronic Liver Diseases

Highlights

- HCV viral load increased with IL-28Bin HCV with or without *S. mansoni*co-infection.
- IL-28B and IFN-γ levels correlate in HCV and *S. mansoni*/HCV co-infected patients.
- IL-28B is higher in *S. mansoni*/HCV co-infected patientsthan mono-infected.
- We arethe first to report that IL-28B is significantly higher in *S. mansoni*/HCV co-infectionthan mono-infection.
- L-28B plasma levels; a novel prognosis biomarker in *S. mansoni*/HCV co-infection.

Abbreviations

ALP: Alkaline Phosphatase
ALT: Alanine Trans-Aminase
AST: Aspartate Trans-Aminase
IFN-γ: Interferon-gamma
IL-28B: Interleukin-28B
IHAT: Indirect Hemagglutination Technique
γ-GT: Gamma Glutamyl Transferase
Treg: Regulatory thymocytes
Th1: T-helper 1 cells

Introduction

Intestinal schistosomiasis caused by *S. mansoni* is endemic in Egypt and so is HCV infectionswith highest prevalence worldwide 10-30% [1]. HCV infection in Egypt is usually associated with *S. mansoni* co-infection causing severe liver damage [2,3]. Severe liver damage in patients with co-infection might be due to failure to produce efficient HCV-specific immune responses leading to persistence of HCV infection and liver damage [4]. IFN-γ, a Th1 cytokine, is associated with both diseases. IL-28Bis part of the interferon-stimulated gene products (ISGs) [5] that are implicated

in resistance of many pathogens including viral and parasites. IL-28B gene polymorphism is linked to variation in response to HCV therapy [6]. However, little is known about IL-28B levels in chronic *S. mansoni*/HCV patients and correlation with severity of disease. Therefore, we aimedto examine levels of endogenous IL-28B in naïve HCV patients with or without *S. mansonico*-infection as a possible novel biomarker for prognosis and therapy.

Methods

Subjects included in this studyhad no history of treatment with pegylated-IFN or any anti-HCV therapy. Exclusion criteria included serious co-morbid conditions such as heart diseases, poorly controlled diabetes or chronic obstructive pulmonary diseases. Patients with known HIVco-infection, solid transplant organ, untreated thyroid disease, alcoholic liver disease, hemochromatosis, α-1 antitrypsin deficiency, or Wilson's disease were excluded from this study.

Liver function tests

Plasma levels of AST, ALP, ALT, total and direct bilirubin were assayed using colorimetric kits from Quimica Clinica Aplicada, S.A. (QCA) (Tarragona, Spain).γ-GTplasma levels were assayed using colorimetric assay (Reactivos GPL, Spain). Total plasma protein was assayed using biuret reaction method while albuminwas assayed using bromocresol green reaction (BCG) both kits from (Stanbio Laboratory, Texas, USA).

Viral assays

Qualitative HBsAg test performed with ABON HBsAg Rapid Test (Abon Biopharm Co., Ltd., China). Total viral RNA was isolated from patients' plasma using QIAamp viral RNA kit (Qiagen, Germany) following manufacturer's protocol. We used real-time PCR for measuring HCV viral load using Quanti Tect Probe RT-PCR Kit (Qiagen, Germany) as previously described [7] positive results >10 cpm.

S. mansoni infection assays

S. mansonieggs were detected inschistosomiasis patients' feces using rectal snips. Plasma anti-schistosomal antibody titers were assayed for all subjects using indirect hemagglutiation kit (Fumouze Diagnostics, France). Titers > 1:160 were considered positive.

Cytokine assay

IFN-γ plasma levels were assayed using solid phase enzyme amplified sensitivity immunoassay DIAsource IFN-γ-EASIA (DIAsource Immunoassays, Belgium). IL-28B levels in plasma were measured using ELISA kit for human IFN-lambda-3 (EIAab Co., Ltd., China).

Statistical analysis

All statisticalanalysis was performed using GraphPad Prism v6.01(California, USA). Nonparametric Mann-Whitney t-test was performed to calculate the differences between groups and data were expressed as medians ± standard error (SE). p-value< 0.05 for all statistical significance tests.

Results

Serum total protein and albumin were significantly lower in *S. mansoni*/HCV co-infected patients than in mono-infected group ($p<0.01$) (table 1). AST, ALT, and γ-GT were higher in *S. mansoni*/HCV co-infection than HCV mono-infection ($p<0.0001$)(table 1). All subjects displayed negative HBsAg and HCV viral loads were non-significantly different between HCV mono- and co-infected patients. IL-28B levels were significantly higher in *S. mansoni*/HCV co-infected patients ($p<0.05$) (table 1). IFN-γ was non-significantly different between HCV mono- and co-infected groups (Table 1).

Table 1: Demographics, HCV viral load, *S. mansoni* titer, plasma levels of liver enzymes and cytokines in chronic HCV and HCV / *S. mansonico*-infected patients in comparison to healthy controls

Parameters	Healthy controls n = 35	Chronic HCV patients n = 50	S. mansoni / HCV co-infected patients n = 22
Median age ± SE (range)	41.0 ± 0.7 (32–47)	44.5 ± 1.6 (30–81)	43.5 ± 1.5 (31–51)
Gender (male : female)	18 : 17	23 : 27	16 : 6
HCV load (x 10^4cpm) ± SE	8 ± 0.3	63.8 ± 42.9[a]	60.0 ± 24.1[b]
anti-S. mansoni titer± SE (range)	80.0 ± 5.8 (80–160)	160.0 ± 5.7 (80–160)	320.0 ± 87.8[b,c] (320–1280)
Liver profile			
AST (IU/L) ± SE	27.0 ± 0.6	270.5 ± 4.8[a]	312.5 ± 7.6[b, c]
ALT (IU/L) ± SE	17.0 ± 0.3	240.5 ± 4.5[a]	292.0 ± 6.0[b,c]
AST/ALT ratio	1.7 ± 0.04	1.1 ± 0.01 [a]	1.1 ± 0.01 [b,c]
γGT (IU/L) ± SE	15.0 ± 0.4	123.8 ± 3.2 [a]	230.6 ± 9.0 [b,c]
ALP (IU/L) ± SE	87.0 ± 2.1	282.6 ± 9.5[a]	327.2 ± 15.4 [b]
Total Protein (g/dl) ± SE	6.8 ± 0.1	4.8 ± 0.1[a]	3.4 ± 0.2 [b, c]
Albumin (g/dl) ± SE	4.9 ± 0.04	3.0 ± 0.04[a]	2.1 ± 0.1 [b,c]
Total Bilirubin (mg/dl) ± SE	0.2± 0.004	4.6 ± 0.5[a]	3.8 ± 0.5 [b]
Direct Bilirubin (mg/dl) ± SE	0.1± 0.003	2.3± 0.3[a]	2.0± 0.2 [b]
IFN-γ (IU/ml) ± SE	0.1 ± 0.005	0.3 ± 0.01[a]	0.3 ± 0.02 [b]
IL-28B (pg/ml) ± SE	6.2 ± 0.3	631.5 ± 67.6 [a]	918.9 ± 83.8[b, c]

[a] Statistical significant difference between chronic HCV patientsand healthy control groups ($p<0.0001$),
[b] Statistical significant difference between *S. mansoni*/ HCV co-infected patients and healthy controls groups ($p<0.0001$),
[c]Statistical significantdifference between chronic HCV and *S. mansoni*/ HCV co-infected patients ($p<0.05$)

[d] data expressed as medians ± standard error (SE)

Correlation analysis showed that IFN-γ levels were positively associated with IL-28B levelsin mono-infection (r = 0.95, p<0.0001) and co-infection (r = 0.87, p<0.0001) (figure 1). IL-28B levels were positively associated with HCV viral load in mono-infection (r = 0.813, p<0.001) and co-infection (r = 0.89, p<0.001) (figure 1). Similarly, IFN-γ levels were positively associated with HCV viral load in mono-infection (r = 0.897, p<0.001) and co-infection (r = 0.949, p<0.001) (Figure 1).

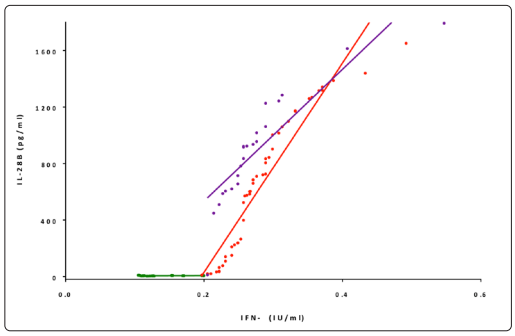

Figure 1: Correlation analysis between levels of IL-28B levels (pg/ml) and endogenous IFN-γ levels (IU/ml) showed positive association in chronic HCV patients (red dots and regression line) (r = 0.99, p< 0.001) and *S. mansoni*/HCV co-infected patients (purple dots and regression line) (r = 0.99, p< 0.001), but not in healthy controls (green dots and regression line) (r = 0.20, p> 0.05), where r is Pearson's Product-moment correlation coefficient.

Discussion

One of the factors modulating the different outcomes of *S. mansoni*/HCV is Th1/Th2 balance along with Th17 and Treg responses [3]. Our study showed a significant elevation of Th1 IFN-γ in HCV subjects with or without *S. mansoni* co-infection compared to controls. This can be attributed to the dominance of Th1 immune responses to HCV [8]. We have shown that IL-28B and IFN-γ levels are directly correlated in HCV patients with or without *S. mansoni* co-infection. This is similar to observed in influenza viral infection, whereIL-28B and IFN-γ were found to have astrong association[9]. IL-28B and IFN-γ werealso strongly associated with HCV viral load. This is consistent with a recent study which correlates IL-28B serum levels with the degree of HCV infection [10]. We observed significant elevation of IL-28B in *S. mansoni*/HCV co-infected patients, thismight be a compensation mechanism to the dominating Th2 immune response to *S. mansoni* [8]. Although IL-28B gene polymorphism is used in predicting outcomes of HCV treatment [6], it has not been useful in predicting therapy outcomes in *S. mansoni*/HCV patients [11]. Here we show that IL-28B plasma levels rather than gene polymorphism is a possible novel biomarker for predicting therapy outcomes in *S. mansoni*/HCV patients. Further studies on relationship of IL-28B to balance of Th1, Th2 and Tregsresponses in naïve HCV patients with or without *S. mansoni*co-infection would be useful.

Conclusions

This is the first report to show a strong association between IFN-γ and IL-28B in naïve chronic HCV patients with or without S. mansonico-infection. IL-28B is only affected in HCV patients co-infected with S. mansoni, suggesting association of S. mansoni-with increased IL-28B levels. Further studies should investigate the possibility of using IL-28B as a novel biomarker for therapy in *S. mansoni*/HCV co-infection.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Conflict of interest statement: The authors have no conflict of interest.

Ethics approval: The research ethics committee of the Faculty of Pharmacy, Cairo University, Egypt, approved this study, protocol number MI(522).

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