Assessment of the role of interleukin 17A and interleukin 17F in chronic hepatitis C virus infection in Egyptian patients

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Received 22 December 2018
Accepted 5 January 2019

The Egyptian Journal of Internal Medicine
2019, 31:199–202

Background/purpose
of the study Chronic hepatitis C virus (HCV) infection is considered one of the major health problems. About 170 million patients were infected with HCV worldwide. Till few years, Egypt was considered the highest HCV prevalent country worldwide, with predominant genotype number 4. The host immunity plays a major role in HCV infection with evolving data confirming the role of T-helper 17 cells in the formation of chronic HCV infection. The aim of our work was to determine the role of interleukins 17A (IL17A), 17F (IL17F) in the formation of chronic hepatitis C infection.

Patients and methods
We classify the patients into two groups: the first group included 51 chronic HCV patients who did not take antiviral therapy (the study group) and the second group included 51 healthy blood donors (as a control group). The levels of IL17A and IL17F in the serum of both groups were measured using the sandwich enzyme-linked immunosorbent assay method.

Results
The serum values of IL17A were higher in patients with chronic HCV than the other group with the mean values being 52.9±32.6 pg/ml in the patient group and 17.1 ±10.4 pg/ml in the control group. IL17F was slightly higher in the HCV patient group than the control group, but it was statistically insignificant. Moreover, there were significant positive correlations between IL17A and alanine aminotransferase, viral load, and degree of liver fibrosis.

Conclusion
Patients with chronic HCV infections had a higher serum level of IL17A than the normal persons and it is positively correlated with alanine aminotransferase, viral load, and degree of liver fibrosis. This suggests its pivotal role in the formation of chronic HCV infection; so, it can be used as a new marker for disease progression due to its positive correlation with the severity of liver injury.

Keywords:
hepatitis C virus, immune response, interleukin 17, T-helper cells

Egypt J Intern Med 31:199–202

1110-7782

Introduction

Chronic hepatitis C viral infection is considered one of the major health problems around the world. About 170 million persons are chronically infected with hepatitis C virus (HCV). Chronic HCV can result in progressive hepatic injury, fibrosis, cirrhosis, end-stage liver diseases, and risk of hepatocellular carcinoma [1]. Egypt has a high prevalence of HCV worldwide, which can be explained by the past practice of parental therapy for schistosomiasis, absence of infection control procedures in many health practices [2]. The immunity against HCV infection has a unique mechanism because it is not only involved in viral clearance but also lead to liver cell affection. HCV balances this way by wreaking both adaptive and innate immune responses leading to a reduction of viral clearance and immune-mediated hepatic injury [3]. The host immunity to HCV infection was mediated by cellular and humoral immunity. Both $CD4^+$ T and $CD8^+$ T cells play vital roles in the process of HCV-related immune response [4]. Th17 cells was mentioned as a novel subgroup of the specialized T-helper (Th) cells which produce several cytokines (IL17A, IL17F, IL21, and IL22) and are powerful enhancers of tissue-related inflammation. It requires TGFβ and other cytokines such as IL6 and IL23 for their differentiation [5]. The IL17 is a group of cytokines contributed in many inflammatory responses and has a role in the formation of multiple inflammatory disorders. There are six members in this family: IL17A, IL17B, IL17C, IL17D, IL17E (or IL25), and IL17F with IL17A and IL17F being the most nearly related members of this group. Although

IL17A production can initiate host defense and neutrophil accumulation, its pathological formation can cause marked inflammation and excessive tissue damage [6]. Th17 and interleukin 17 (IL17) appear to have a vital function in the pathogenesis of viral infections and could also play a role in hepatic viral persistence by means of upregulation antiapoptotic molecules [7]. The aim of our work was to clarify the role of Th17 cells cytokines, IL17A and IL17F in the pathogenesis of chronic infection with HCV.

Patients and methods

This work was carried out in the Hepato-gastroenterology Unit of Internal Medicine Department in collaboration with Microbiology and Immunology Department, Zagazig University, Egypt during the period from May 2016 to May 2018. The study included 102 subjects who were divided into two groups. Group A (patients' group) included 51 nontreated chronic HCV patients who attended the HCV treatment at the hepatitis viruses' management center in Al-Ahrar Hospital (Zagazig, Egypt). Diagnosis of chronic HCV was by standard diagnostic criteria (positive anti-HCV antibodies by enzyme-linked immunosorbent assay (ELISA) with positive HCV RNA by PCR for more than 6 months. Group B (control group) included 51 apparently healthy blood donors who were negative for anti-HCV antibodies, HbsAg, and anti-HIV antibodies by ELISA. Both groups were of matched age and sex with the patients' group. Patients were excluded if they had any chronic medical disease, other causes of liver disease such as alcoholic liver injury, hepatitis B, autoimmune liver disease, hemochromatosis, Wilson's disease, α1 antitrypsin deficiency, or if they had received any treatment for HCV before. According to declaration of Helsinki.

Methods

All subjects of the study were subjected to the following: full history and thorough physical examination to exclude any chronic disease. Laboratory investigations such as complete blood picture by automated blood counter (Sysmex KX-21), liver function tests: serum bilirubin (total and direct), serum albumin, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) measured by the kinetic method by COBAS INTEGRA 400 plus (ROCHE INTEGRA 400 PLUS, Roche Diagnostics), renal function tests: serum creatinine, urea by COBAS INTEGRA 400 plus, coagulation profile: prothrombin time, partial thromboplastin time, international normalized ratio, and PC by Sysmex CA-1500 (The Sysmex® CA-1500 SIEMENS HEALTHINEERS, USA), viral marker for HCV, HBV, and HIV by ELISA by STATFAX 3000 (FibroScan ,Ohio State, USA), HCV RNA by PCR for the patient group (Taqman Real Time PCR). Pelvi-abdominal ultrasonography, transient elastography (FibroScan): to all patients group to assess the stage of hepatic fibrosis. Elastometry correlated with METAVIR score system as follows: 0–2.9 kPa by elastometry correlated for F0 at METAVIR score, 3–5.9 kPa for F1 score, 6–8.9 kPa for F2 score, 9–16.9 kPa for F3 score, and 17–75 kPa for F4 score [8]. Special investigation: measurement of IL17A and IL17F for both patient and control groups by using eBioscience→Human IL17A Platinum ELISA and eBioscience→Human IL17F Platinum ELISA kits. Three milliliters of fresh venous blood was collected in an anticoagulant-free disposable plastic tube after skin sterilization and incubated at room temperature (20–27°C) until clot retraction and separation of the serum. Then, these tubes were centrifuged at 1000 rpm for 15 min for good separation of serum and the obtained serum was collected in two Eppendorf tubes and was stored in deep freeze at −20°C.

Results

The mean serum level of IL17A was higher in the case group than the control group with the level ranging from 11 to 150 pg/ml in the case group and 7–50 pg/ml in the control group with high statistically significant difference between the two groups as the *P* value is less than 0.0001 (Tables 1 and 2).

Also, the mean serum level of IL17F was higher in the case group than the control group with the level ranging from 4 to 198 pg/ml in the case group and 4–34 pg/ml in the control group, but with no statistically significant difference between the two groups as *P* value is more than 0.05 (Table 3).

When we do one-way analysis of variance test to correlate the fibrosis stage with the level of both

Table 1 Demographic data of the enrolled patients (group A)

	Age	Viral load	ALT	AST	Total bilirubin	Lymphocyte %	Duration of disease (months)
Mean	41.5882	917 449.8431	55.7843	35.5294	0.7686	28.3627	56.352
SD	9.93816	107 535.9755	36.83874	20.9536	0.43774	5.73679	24.215

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 2 Difference in the serum levels of interleukin 17A between case and control groups

IL17A	Case group	Control group	*t*	*P*
Mean	52.9216	17.1765	6.7	0.0001
SD	32.62443	10.49896		

IL17A, interleukin 17A.

Table 3 Difference in the serum levels of interleukin 17F between case and control groups

IL17F	Case group	Control group	*t*	*P*
Mean	18.9804	14.6863	0.8	0.25
SD	26.30170	6.14976		

IL17F, interleukin 17F.

Table 4 Fibroscan results findings of the patients' group

Fibrosis stages	IL17A (mean±SD)	IL17F (mean±SD)
F0–F1	46.12±5.27	27.44±3.6
F2	76.29±6.03	26.73±4.77
F3	95.24±7.42	25.4±3.47
F4	138.64±7.17	20.59±2.32
One-way ANOVA	555.785	8.295
P	<0.001	<0.1

ANOVA, analysis of variance; IL17A, interleukin 17A; IL17F, interleukin 17F.

Table 5 Correlation between viral load and serum levels of both interleukins 17A and 17F among the patients' group

Groups	Viral load
IL17A	
r	0.883
P	0.000
IL17F	
r	–0.163
P	0.252

IL17A, interleukin 17A; interleukin 17F.

IL17A and IL17F, there was a statistically significant association between the stage of fibrosis and IL17A levels but nonsignificant difference with IL17F level with a *P* value of less than 0.001 and less than 0.1, respectively (Table 4).

There were strong positive correlation between levels of IL17A (range, 11–150 pg/ml) and viral load (range, 14 998–5 431 737 IU/ml) and this correlation was statistically highly significant (r=0.883, P<0.001).

On the other side there was a weak negative correlation with IL17F (range, 4–198 pg/ml), but this correlation was statistically nonsignificant (r=–0.163, P>0.05) (Table 5).

There were strong positive correlations of patients' serum level of IL17A (range, from 11–150 pg/ml), with both ALT (range, 11–172 U/l) and AST (range, 11–91 U/l) and these correlations were highly statistically significant (r=0.523, P<0.001 and r=0.587, P<0.001, respectively).

Table 6 Correlation between liver function tests and serum levels of both interleukins 17A and 17F among the patients' group

Groups	ALT	AST	Total bilirubin
IL17A			
r	0.523	0.587	0.103
P	0.000	0.000	0.470
IL17F			
r	0.125	0.159	0.170
P	0.383	0.265	0.232

ALT, alanine aminotransferase; AST, aspartate aminotransferase; IL17A, interleukin 17A; interleukin 17F.

On the other hand, there were negative correlations between IL17F and both ALT and AST, but this was nonsignificant. There were weak negative correlations between serum bilirubin and both IL17A, F level; however, it was nonsignificant (Table 6).

Discussion

HCV is one of the major causes of chronic hepatitis worldwide, affecting about 175 million people [8]. Nearly two-thirds of HCV-infected patients attain chronic disease that leads to hepatocellular carcinoma and end-stage liver disease [9]. HCV modulates the immune system of the infected patient [8]. T cells have a vital role in viral elimination [10]. Conversely, the virus usually persist, together with disturbed effector T-cell responses [11]. T cell is the most involved cell lineage in HCV immune-mediated response. T $CD8^+$ and Th1 are concerned with the control of viral replication. Some reports have verified that Th17 cells have been concerned with either pathogenesis or protection of HCV infection. Th17 cells are a proinflammatory T-cell subset which is newly identified, which is different from Th2 and Th1 cells by their formation of IL17A, F, IL22, IL21, and IL26. Also [12] Th17 cells were considerably elevated in patients with chronic HCV, which were considered to be crucial factors for triggering liver inflammation [13]. IL17 is formed and secreted by activated $CD4^+$ T cells which then produces a wide range of inflammatory immunological effect [14]. In our study, the serum levels of IL17A was significantly higher in chronic HCV patients' group with the mean 52.9 ±32.6 pg/ml than in the control group with the mean 17.1±10.4 pg/ml. IL17F is known to be a weak initiator of proinflammatory cytokines which is released by Th17 cells and induces immune defense against infection by inducing the formation of chemokines, Granulocytic colony stimulating factor (G-CSF) and antimicrobial

peptides. In this work, IL17F was also slightly higher in the chronic HCV group with the mean being 18.9 ±26.3 pg/ml than the other group. The mean was 14.6 ±6.1 pg/ml although this elevation was statistically nonsignificant. These results came in agreement with the Lemmers *et al.* [15] study which showed that the serum level of IL17 and $IL17^+$ cells were elevated in patients with chronic HCV infection. Other studies also declared that the plasma levels of IL17 were high in patients with alcoholic hepatitis and cirrhosis [16], and the serum levels of IL17 and Th17 cells increase in the blood in patients with chronic HBV [17].

IL17A was strongly positively correlated with AST, ALT levels. These results match the results of Cachem *et al.* [12]. This is also in agreement with the Zhang *et al.* [17] study which found a positive correlation between Th17 cells and ALT. Chang *et al.* [18] also showed that IL17A positively correlated with ALT level, which came in agreement with our results. There were negative correlations between IL17F with both ALT and AST, but this was not significant. There was a weak negative correlation between serum bilirubin and both IL17A, IL17F; however, it was not significant.As regards viral load, our study showed a strong positive correlation between IL17A and HCV RNA and it was highly statistically significant. Also, there was aweak negative correlation between IL17F and viral load, but this correlation was statistically nonsignificant. These data were not proven by many studies as in Chang *et al.* [18]. Sousa *et al.* [19] observed that there were no correlation between IL7 and viral load.

Also, the IL17A levels were positively correlated with the degree of liver fibrosis measured by Fibroscan with higher levels of IL17A being in patients with F3, F4 fibrosis. IL17F values were slightly higher in early fibrosis stages F1, F2, than F3, F4 but it was statistically insignificant.

The observation that the IL17F was lower in HCV-infected persons requires further research. IL17F downregulates IL6, IL8, and vascular endothelial growth factor and inhibits angiogenesis and cancer growth [20], so it may have a protective role in HCV-infected persons.

Conclusion

The serum level of IL17A was higher in chronic HCV patients' group suggests its role in the pathogenesis of chronic HCV infection and its positive correlation to ALT, viral load, and the degree of liver fibrosis can use it as a new marker for disease prognosis. On the contrary, IL17F was negatively correlated with HCV disease severity suggesting that it may have a protective role.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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