**The Role of IL-17 in the Pathogenesis of HCV and Schistosomiasis Co-Infection**

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**Abstract: Aim of the study**: The present study was conducted to evaluate the role of IL-17 in the pathogenesis of HCV and schistosomiasis co-infection. **Patients and methods**: A prospective analysis was done for 51 patients in hepatology unit in El-fayoum general hospital, who received therapy from March 2016to September 2016. Our patients were divided into four groups our patients in combined schistosoma and HCV group and HCV group received the anti-viral treatment. IL-17 cytokines was quantitated by ELISA at the start and end of therapy. **Results**: eight positive patients (16%), of whom six patient was diagnosed as *Schistosoma haematobium* infected and two patients as *Schistosoma mansoni* infected in comparison to thirty three patients (60%) with using ELISA. Higher levels of IL-17 among group of combined *schistosoma* and HCV group (524.2±168) in comparison to group of *Schistosoma* alone (326±39.2). following treatment, there was statistically significant decrease with p-value <0.05 in IL-17 level after treatment among each *Schistosoma* with HCV (241.4±67.1) and HCV group (154.5±65.7); which indicated that both groups are simultaneously responded to treatment. **Conclusion:** schistosomiasis may not affect the outcome of HCV infection in genotype 4-infected patients but *Schistosoma* infection might aggravate HCV-related liver disease through induction of changes in the regulatory T-cell phenotype through increase IL-17 level.

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**Key words:** *Schistosoma*, IL-17, HCV, anti-*Schistosomal* antibodies, HCV drugs.

**1. Introduction**

Schistosomiasis is the second most important parasitic infection after malaria and affects more than 200 million people in 74 countries (***WHO, 2002***). In Egypt, according to Ministry of Health records, By the end of 2010, only 20 villages had prevalence more than 3.5% and none had prevalence more than 10% ***(WHO, 2011).*** It has become evident that the parenteral administration of tartar emetic using non sterilized syringes resulted in widespread transmission of both HCV and HBV **( El-Sabah et al.,2011).** Given the prevalence of schistosomiasis/HCV coinfection in Egyptians, higher susceptibility of this population was proposed. **(Elsammak, et al., 2008).**

**Aim of the work**

The present study was conducted to evaluate Role of IL-17 in the pathogenesis of HCV and schistosomiasis co-infection**.**

**2. Patients, Materials and Methods**

**Study population**

A prospective analysis was done for 51 patients in hepatology unit in El-fayoum general hospital, who received therapy from March 2016to September 2016. They were classified into four groups, our patients in group I and group II received treatment in the form of: Sofosbuvir 400 mg tab. and daclatasavir 60 mg daily for 3 months. All subjects participated in the study signed written informed consents. The study was approved by the research ethics committee of Faculty of medicine, Fayoum University, Egypt. They were classified into four groups: Group I (n= 25):: patients with concomitant schistosomiasis and chronic HCV infection. inclusion criteria were as follows: seropositivity for antibody to HCV, positive for HCV RNA by PCR, elevated aminotransferase levels for more than 6 months, (Ultrasound) showing evidence of chronic hepatitis and the Patients had active schistosomiasis or had chronic infection (with a long history of schistosomiasis, repeated exposures, positive S. mansoni antibodies with either viable or nonviable ova in stool or urine), and no current or previous therapy with interferon (IFN) or ribavirin. Group-II (n=8): patients with chronic HCV infection. Group III (n=10): patients had active schistosomiasis Or had chronic infection with history of schistosomiasis exposure, detection of Schistosoma ova in stool or urine or seropositivity for Schistosomal antibodies (indirect heamagglutination). Group-IV (8): healthy individuals as controlsAll studied cases were subjected to Full history taking and clinical examination **Cytokine measurements.** Serum cytokines was quantitated by ELISA for IL-17. Follow up and monitoring of cytokines level (IL-17 A) was done at the start and end of therapy. Procedure: Microwell strips required to test the samples plus wells needed for running blanks and standards were prepared and assayed in duplicate. Extra microwell strips were removed from holder and stored in foil bag with the desiccant provided at 2°-8°C sealed tightly. The microwell strips were washed twice with approximately 400 µl Wash Buffer per well with thorough aspiration of microwell contents between washes. The Wash Buffer was allowed to sit in the wells for about 10 – 15 seconds before aspiration. Takeen care not to scratch the surface of the microwells. After the last wash step, wells were emptied and microwell strips were tapped on absorbent pad or paper towel to remove excess Wash Buffer. The microwell strips were used immediately after washing. 50 µl of Sample Diluent was added to all wells.50 µl of extern prepared standard dilution was added in duplicate to designated standard wells. 50 µl of Calibrator Diluent in duplicate to the blank wells.50 µl of each sample in was added in duplicate to designate sample wells. Biotin-Conjugate was prepared.50 µl of Biotin-Conjugate was added to all wells. Covered with an adhesive film and incubated at room temperature (18 to 25°C) for 2 hours on a microplate shaker. Streptavidin-HRP was prepared. Adhesive film was removed and wells were emptied. microwell strips were washed 6 times.100 µl of diluted Streptavidin-HRP was added to all wells, including the blank wells. Covered with an adhesive film and incubated at room temperature (18° to 25°C) for 1 hour on a microplate shaker. Adhesive film was removed and wells emptied. Microwell strips were washed 6 times. 100 µl of TMB Substrate Solution was pipetted to all wells. The microwell strips were incubated at room temperature (18° to 25°C) for about 30 min. direct exposure to intense light was avoided. The colour development on the plate was monitored and the substrate reaction was stopped before positive wells are no longer properly recordable.

The substrate reaction was stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95. The enzyme reaction was stopped by quickly pipetting 100 µl of Stop Solution into each well. Results were read immediately after the Stop Solution is added. absorbance of each microwell was read on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable).

**3. Results**

In the present study, with using direct parasitological methods (urine and stool analysis), we detected eight positive patients (16%), of whom six patient was diagnosed as *Schistosoma haematobium* infected and two patients as *Schistosoma* *mansoni* infected in comparison to thirty three patients (60%) with using IgG EIA. In this study, regarding group III (*Schistosomal* group), frequency of infection among patients showed difference in sex distribution with prevalence of infection among males (60%) more than females (40%). This study as shown in Table (1 and 2) demonstrated a high level of infection in 2 age groups; 44.9±14.8 and 51.4±12.6 years old. In the present study, 25 of patients ( 50%) had HCV and schistosomiasis co infection. In our study, there is statistically significant difference with p-value <0.05 between different study groups as regards IL-17 level before treatment with low means were noticed among controls (**76.9±3.4**), high means were among group of *Schistosoma* **(326±39.2)** and higher levels among group of combined *Schistosom*a and HCV (**524.2±168**).

**Table (1): Comparisons of demographic characters in different study groups.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variables** | **Schistosoma with HCV**  (n=25) | **HCV**  (n=8) | **Schistosoma**  (n=10) | **Control**  (n=8) | **p-value** | **Sig.** |
| **Age (years)** | | | | | | |
| Mean /SD | 44.9±14.8 | 48±11.1 | 51.4±12.6 | **33.8±6.1** | **0.04** | **S** |
| **Sex** | | | | | | |
| Males | 15(60%) | 3(37.5%) | 6(60%) | 3(37.5%) | 0.5 | NS |
| Female | 10(40%) | 5(62.5%) | 4(40%) | 5(62.5%) |

**Table (2): Comparisons of Parasitological investigations.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variables** | **No. Patient examined** | **+ Ve Cases** | **%** |
| **Stool analysis** | **51** | **2 *(S. mansoni*)** | **3.92** |
| **Urine analysis** | **51** | **6(S. *haematobium)*** | **11.76** |
| **Total** | **51** | **8** | **15.68** |

**4. Discussion**

Chronic infection with hepatitis C virus (HCV), the second most important emerging infection and possibly the most important worldwide cause of liver disease, is estimated to be present in 170 million people. Egypt has the highest prevalence of HCV, being 10–25% in most of the same rural areas where schistosomiasis is endemic **(EL-Khoby, et al., 2001).** The present study was conducted, to evaluate role of IL-17 in the pathogenesis of HCV and schistosomiasis co-infection. Interleukin-17 may be a major contributor to schistosomiasis-associated liver pathology **(Rutitzky et al., 2011**). being the most associated with the progression of cirrhosis, There are receptors for IL-17 expressed in hepatocytes, in the liver sinusoids endothelial cells, in hepatic stellate cells (HSCs), and Kupffer cells (KC) The functional implication of IL-17 in liver tissue is well characterized in the activation and/or stimulation of HSCs and KC **(Meng et al., 2012).**

In our study, there is statistically significant difference with p-value <0.05 between different study groups as regards IL-17 level before treatment with low means were noticed among controls (**76.9±3.4**), high means were among group of *Schistosoma* **(326±39.2)** and higher levels among group of combined *Schistosom*a and HCV (**524.2±168**).

This indicates that presence of HCV infection shifted the response towards Th17 pathway which may play a role in the progressive pathogenesis of HCV in *Schistosoma*-co-infected patients.

This is in accordancewith **Nady et al. (2016)** stated that There was a significant increase in plasma levels of IL-17 in co-infected patients compared to *Schistosoma*-infected patients.

Moreover, **Dianhui et al. (2013)** in vitro release was substantially higher in lymphocytes from infected livers (IL-17: 1·14 ± 0·12 ng/ml), suggesting that the production of IL-17 in the liver is markedly enhanced by infection (*P* < 0·05)**.**

**Smith et al. (2009)** Interleukin-17 expression by lymphocytes is a marker of disease severity in schistosomiasis.

**Kryczek et al. (2007)** Interleukin-17-producing T cells, including **γδT** cells, NKT cells and CD4+ Th cells, have been implicated in the development of granulomatous disease**.**

This is consistent with IL-17 release which showed a significantly higher percentage of T cells from infected livers expressed these cytokines following activation. Moreover, in parallel with the result obtained with CD3+ cells, the proportion of IL-17+ cells increased after infection ( IL-17: 1·49 ± 0·85% versus 0·35 ± 0·17%, P < 0·01,). Additionally, the proportions of both IL-17+ CD3+  cells were significantly higher than of CD3− cells in both normal control and infected group (P < 0·05). **(Dianhui et al., 2013).**

In the present study, IL-17 levels were significantly markedly reduced following treatment in anti *Schistosoma* anti-bodies positively patients as shown in Figure (1) and Figure (2).

As the evolving cellular response to S. japonicum infection is gradually accompanied by production of non-complement fixing IgG and IgE antibodies**. (Gause et al., 2003),** Hence, IL-17 normally serves to suppress SEA-specific IgG expression, which is consistent with the results of **Wen et al. (2011)**.



Figure (1): sex in different study groups



Figure (2): Mean IL-17 level before treatment in different study groups

Although the functions of Th17subset are not completely understood, several studies suggested the important role of Th17 cells in host defenses against extracellular pathogens and in the immunopathogenesis of some infectious diseases by producing their crucial regulatory cytokine IL-17 **(Grzych et al., 1991; Rutitzky et al., 2008; Rutitzky et al., 2009; Rutitzky & Stadecker, 2011; Shainheit et al., 2011; Smith et al., 2009).** **Wen et al. (2011)** reported that Th17 cells are directly associated with the severity of hepatic egg-induced granulomatous inflammation.

The level of Th17 cells in the host is determined by multiple factors including exposure to complex parasitic antigens that either induce or suppress the generation. Lowering IL-17 levels may also favor the host’s protective responses against *Schistosoma* infection **(Shainheit et al., 2011; Wen et al., 2011).** An increase in the frequency of Th17 cells located in the liver granulomas and spleens of *Schistosoma*-infected mice was reported which could be reduced by neutralization of IL-17 in vivo **(Mbow et al., 2013; Rutitzky et al., 2005; Rutitzky et al., 2008).** Patients co-infected with HCV and schistosomiasis exhibit unique clinical, virological, and histological patterns manifested by viral persistence with high HCV RNA titers, as well as higher necro-inflammation and fibrosis in the liver **(Angelico et al., 1997; Kamal et al., 2000b).**

**Fathy et al., (2011)** reported that Th1 responses have been associated with protective host defense against HCV.

**Balanescu et al., (2012); van de Veerdonk et al., ( 2009)** recently suggested that HCV can also induce Th17 cells, although their role in antiviral host defense still unclear.

**Van de Veerdonk et al., (2009)** reported that the type of microorganisms and the associated microenvironment in which they trigger the Th17 response determine the outcomes of the disease and the balance between Th17 induced protection and immunopathogenesis.

**Loffredo-Verde et al., ( 2015)** stated that *Schistosoma* infection might aggravate HCV-related liver disease through induction of changes in the regulatory T-cell phenotype.

Our results are in accordance with previous studies which demonstrated an increase in circulating Th17, intrahepatic IL-17 positive cells, as well as HCV-specific Th17 cells. The increase in IL-17 was correlated with severity of liver inflammation in chronic HCV infected patients **(Balanescu et al., 2012; Fathy et al., 2011).**

As regard group (II), IL-17 levels were elevated in the serum of chronic HCV patients and values did not correlate with viral loads following 12 weeks of treatment, IL-17 levels showed significant reduction and this was in line with **Jimenez-sousa et al., (2010).**

On the **contrary Fathy et al., (2011)** found that serum IL-17 cytokine was not reduced.

**Conclusion**

Our data indicated that HCV infection amplified the Th17 bias during *Schistosoma* co-infection which may play a role in the progressive pathogenesis of HCV in *Schistosoma* co-infected patients.

This study significantly contributes to our understanding of immunity to schistosomiasis and HCV co-infection and may aid in developing intervention tools to protect hosts from infection or restrain the immunopathogenesis of co-infection.

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