Th17 Cytokines Modulates Neutrophil Response to Co-Infection with *Schistosoma* SEA and HCV Core Protein

Soad Nady and M. Tarek Shata*

***Abstract*—**This study aimed to investigate the role of neutrophils in *Schistosoma*/HCV co-infection in the presence of Th17 cytokines (IL-17 and IL-22). I*n vitro* studies on the effects of SEA antigens of *Schistosoma* and HCV core protein on healthy neutrophil functions and *in vivo* studies on effects of co-infection on plasma IL-17 and IL-22 levels as well as neutrophil mediators; TNF-α and H_2O_2 levels (using ELISA) were included. A significant decrease in TNF-A production and increase in H_2O_2 production by (SEA+core) stimulated neutrophils comparing to SEA or core protein was observed. IL-17 significantly increased TNF-α but not hydrogen peroxide. IL-22 significantly increased TNF-α and decreased H_2O_2. Significant increases in TNF-α and H_2O_2 levels in coinfected plasma. IL-17 levels were significantly higher and IL-22 level was significantly lower in coinfected plasma comparing to *Schistosoma* infected plasma. Conclusively, IL-17 and IL-22 have differential effects on Neutrophil response to co-infection with *Schistosoma* and HCV.

***Keywords*—**Co-infection, Hepatitis C virus, *Schistosoma*, Th17 cytokines.

LIST OF ABBREVIATIONS IN THE ORDER OF APPEARANCE

Soluble egg antigen (SEA)
Hepatitis C virus (HCV)

I. INTRODUCTION

SCHISTOSOMIASIS affects almost 240 million people worldwide. The infection is prevalent in tropical and sub-tropical areas, mostly in poor communities without potable water and adequate sanitation. Egypt is one of the highest endemic areas of schistosomiasis. Additionally, among *Schistosoma* infected populations, hepatitis C virus (HCV) co-infected 20-50% of *Schistosoma*-infected patients in Egypt [1]-[6]. The association between HCV and schistosomiasis in Egypt is due to the mass parenteral anti-Schistosoma treatment [7]. Immunological state and HCV co-infections are important factors for the diverse clinical patterns observed for schistosomiasis [8]-[10].

Patients coinfected with HCV and schistosomiasis exhibit a unique clinical, virological and histological pattern manifested by high HCV-RNA titres, as well as higher levels of inflammation in the liver [11], [12]. Dual infections are associated with significant changes on the host immune responses including cytokine shift pattern alteration, cytotoxic T-lymphocyte response and other impaired immunologic functions with diminished capacity to clear the virus [13], [14]. The interactions between the two infections are complicated but several studies reported that Th17 cells and their products like IL-17, and IL-22 play an important role in the immunopathogenesis of schistosomiasis [11], [12], [15]-[18]. Additionally, recent studies reported that in animal models, lack of IL-17A signaling lead to an ineffective neutrophil response to infections with bacteria, fungi or parasites with ultimate decrease in animal survival [19]-[21]. The aim of this study was to investigate the role of neutrophils in case of *Schistosoma*/HCV co-infection focusing on the HCV core protein and SEA antigens. The relationship between cytokines patterns in the plasma and effect of the HCV core and SEA proteins on neutrophils functions were examined.

This study included two main parts; *in vitro* studies on the effects of SEA antigens of *Schistosoma* and HCV core antigens on neutrophil functions in the presence of Th17 cytokines (IL-17 and IL-22), and *in vivo* studies on the effects of co-infection on the Th17 plasma cytokines levels as well as TNF-α and H_2O_2 levels as functional mediators of neutrophil activation.

II. MATERIALS AND METHODS

A. In Vivo Study

Three groups of patients were enrolled (Table 1): Group 1 included 18 patients infected with schistosoma alone, Group 2 included 50 patients coinfected with chronic HCV and schistosomiasis, and Group 3 included 17 control healthy individuals. Plasma were collected and kept frozen at -20º till performing quantitative ELISA for different cytokines (IL-17, IL-22 and TNF-A). The study was approved by the research ethics committee of Cairo University. All participants gave

Conflict of interest: All authors declare that no conflict of interest exists regarding the work reported in this manuscript.

Financial Support: This investigation was supported by Science and Technology Development Fund (STDF), grant No. 1814 under US/Egypt cooperation program

Soad Nady , Zoology and Entomology Department, Faculty of Science, Helwan University E-mail: soadnady@hotmail.com

M. Tarek Shata* Division of Digestive Diseases, Department of Internal Medicine, University of Cincinnati, OH, USA

informed consent before participation in the study and their demographic data were recorded.

B. In Vitro Study

Isolation of neutrophils from whole blood: Fifteen ml of fresh blood was withdrawn from healthy consenting adults. Neutrophils were isolated from the EDTA anti-coagulated venous blood as described previously [22] with some modifications. Briefly, the blood was centrifuged with Ficol Hypaque (Amersham Pharmacia, Piscataway, NJ) density gradient at 400 x g for 25 min to remove the mononuclear cells. In the cells sediments RBCs were lysed using ACK lysis buffer (Sigma, St. Louis, MO). Resulting neutrophils preparations were >98% pure as assessed by flow cytometry and more than 95% of the neutrophils were viable as measured by trypan blue. Purified neutrophils were washed and re-suspended in DMEM media (Sigma, St. Louis, MO) at the proper concentration.

***In vitro* activation of neutrophils:** *Schistosoma* SEA (Theodore Bilharz Research institute, Giza, Egypt) and LPS-free HCV-related core protein (ViroGen Corp, USA) were used at the optimum concentrations which were 3 µg/ml, and 100 µg/ml for the HCV core protein and *Schistosoma* SEA, respectively. Additionally, the concentrations of IL-17 and IL-22 (R & D Systems, Inc.) were optimized and used at 10 ng/ml and 100 ng/ml respectively.

Measurement of TNF-α production by activated neutrophils using ELISPOT assay: An enzyme-linked immunospot (ELISPOT) assay was performed with TNF-α ELISPOT kit (catalog no. 3510-2H; Mabtech, Sweden) according to the manufacturer's instructions. Briefly, 96-well nitrocellulose-bottomed plates (Millipore, Bedford, MA) were coated with murine anti-human TNF-α MAb at a concentration of 10 µg/ml in PBS and incubated at 4°C. After 24 h, the plates were washed and blocked with RPMI with 10% fetal bovine serum (FBS). Then, neutrophils were added at a concentration of 10^5 cells/well in a 100 µl volume of complete medium (RPMI 1640 containing 10% FBS). For stimulation, *Schistosoma* SEA and LPS-free HCV-related core protein were added according to the experiment protocol. After an 18-h incubation at 37°C and 5% CO2, the plates were washed 5 times with washing buffer (PBS containing 0.5% Tween 20 (Sigma, St. Louis, MO) using an automatic plate washer (Bio Tec Instruments, Inc.). Biotinylated anti-human TNF-α MAb (clone TNF5-biotin) was added at a concentration of 1 ug/ml in dilution buffer (PBS containing 0.05% FBS), and the plates were incubated at room temperature for 2 h. Plates were then washed again five times, and streptavidin-horseradish peroxidase (1:100) in dilution buffer was added. The plates were incubated at room temperature for 1 h. After, five washes with washing buffer, tetramethylbenzidine (TMB) substrate (MabTech) was added. After the spots were developed in 10 to 15 min, the plates were washed with distilled water and air dried. The number of spots was enumerated using an automated ELISPOT 3B analyzer (CTL, Cleveland, OH) [23].

Assessment of H_2O_2 generation by activated neutrophils using flow cytometry: H_2O_2 generation by stimulated neutrophils was measured using dichlorofluorescein diacetate (DCF-DA; Sigma-Aldrich) as previously described [24]. Briefly, isolated Neutrophils (1x 10^6/well) were placed in a 96-well microplate and incubated with the stimuli or controls in a humidified atmosphere of 5% CO2 at 37°C. After 24h incubation, 20 uM/ml of DCF-DA) was added as intracellular reactive oxygen species marker for 20-30 min. Then, cells were washed by PBS and fixed with 1 % paraformaldehyde. Before analysis, cells were washed with PBS and resuspended in PBS. The number of positive cells was measured using flow cytometry (BD) as described previously [24].

Quantitative measurement of plasma cytokines levels and H_2O_2: TNF-α, IL-17 and IL-22 were measured using Human ELISA kits (Booster Immunoleader, USA) according to the manufacturer's instructions. H_2O_2 was measured using colorimetric method (Bio-diagnostic, Egypt) as described [25].

Statistical Analysis: Statistical analysis was performed using paired Student's t test and Graph Pad Prism software. Results with a p value < 0.05 were considered significant.

III. RESULTS

A. In Vitro Results on The Effect of Co-Infection:

As shown in Fig (1A), SEA or core protein induced a significant decrease in the production of TNF-α compared to that produced by non-activated neutrophils. Similarly, there was a marked decrease in TNF-α production by neutrophils stimulated with both SEA and core protein compared to that produced in response to only SEA or core.

Moreover, SEA alone or core protein alone induced a significant decrease in hydrogen peroxide generation by neutrophils as compared to control (Fig 1B). However, both SEA and core protein induced a significant increase in hydrogen peroxide produced by neutrophils as compared to that produced in response to only SEA or core protein.

In the absence of IL-17, SEA and core significantly decreased TNF-α production by neutrophils (Fig 2A). Additionally, there was a marked increase in TNF-α production in the presence of IL-17, and IL-22 as shown in Fig 2A. In contrast, as shown in Fig 2B, H_2O_2 generation by neutrophils in the presence of SEA and core didn't show any significant changes in the presence of IL-17, but it is significantly decreased in the presence of IL-22.

B. In vivo results on the effect of co-infection

68 patients and 17 control healthy individuals were enrolled in the *in vivo* study. The study was approved by the research ethics committee of Cairo University. The patients were recruited from Al-Qaser El-ainy University Hospital. All participants gave informed consent before participation in the study. The demographic data of the enrolled subjects are represented in Table (1). There is no significant difference in the age of males and females within each group (*Schistosoma*

group, co-infected group and control group) or between the different groups as compared to control subjects.

The *in vivo* results were confirmatory to these results. There was a significant increase in TNF-α and hydrogen peroxide levels in coinfected plasma comparing to those in *Schistosoma* infected plasma (Figure 3). In parallel, IL-17 level was markedly higher and IL-22 level was markedly lower in coinfected plasma than those in *Schistosoma* infected plasma (Figure 4)..

IV. Discussion

Schistosomiasis and HCV are endemic diseases in Egypt [26], [27]. Moreover, the prevalence of coinfection with both diseases is high [28], [29]. Neutrophils may play important roles in responses to *Schistosoma*-induced inflammation and HCV infections. Previous studies investigated the cellular immune response to HCV core protein however the response of neutrophils to HCV core has not been studied. Additionally, recruitment of neutrophils by IL-17 has been studied in different inflammatory conditions. However, there is limited studies investigated the role of Th17 cytokines on neutrophil recruitment during schistosomiasis or in case of co-infection. The aim of this study was to investigate the role of neutrophils in *Schistosoma*/HCV co-infection focusing on the HCV core protein and *Schistosoma* soluble egg antigen (SEA) as the main immune modulators in both infections.

The results of the *in vitro* study revealed that neutrophils activated with only SEA or core protein produced a significant decrease in the production of TNF-α (82.6 % and 59.5 %, respectively) compared to that produced by non-activated neutrophils. Similarly, there was a marked decrease in the production of TNF-α by neutrophils stimulated with both SEA and core protein compared to that produced in response to only SEA or core (75% and 89%, respectively). This finding may suggest that SEA and HCV core protein have inhibitory effects on TNF-α production by neutrophils and their effects are agonistic Therefore, coinfection may be associated with decrease in TNF-α production. TNF-α plays significant roles in regulation of the immune responses in different diseases. Several studies have used TNF-A inhibitors to inhibit inflammatory responses associated with diseases like rheumatologic and gastrointestinal diseases [30], psoriasis and psoriatic arthritis [31] as well as neuroinflammatory conditions [32]. Therefore, it is possible that higher HCV viral load observed in coinfected patients is due to suppression of TNF-A production by neutrophils which dampened the immune responses to HCV and leading to high viral load.

Hydrogen peroxide is well known to induce tissue damage when its level increased and not sequestered by antioxidants [33]-[38]. In the current study it was found that SEA alone or core protein alone induced a significant decrease in hydrogen peroxide generation by neutrophils as compared to control. However, there was a significant increase in hydrogen peroxide produced by neutrophils activated with both SEA and core protein by 98 % and 100 %, respectively, compared to that produced in response to only SEA or core protein which further supporting that both SEA and HCV core are agonistic to neutrophils. So, it seems that combined infections will lead increasing H_2O_2 production by neutrophils and expansion of tissue damages produced by *Schistosoma* eggs and HCV infection. However, the mechanism of agonist is not known and is the focus of our current studies.

The decrease of TNF-α with its immune suppressive effect, and increase in the hydrogen peroxide levels with their tissue damage effects may explain the mechanisms of the increase of liver fibrosis and rapid progress to hepatocellular carcinoma in coinfected patients.

Although SEA and core significantly decreased TNF-α production by neutrophils, there were 90% and 80% increases of TNF-α in the presence of IL-17, and IL-22, respectively. In contrast, H_2O_2 generation by neutrophils in the presence of SEA and core didn't show significant changes in the presence of IL-17, but hydrogen peroxide significantly decreased (73 %) in the presence of IL-22. Our results clearly showed that Th17 cytokines, IL-17 and IL-22, not only helps in the recruitment of neutrophils as reported by several studies [11],[39], but differentially modulated the response of neutrophils to both *Schistosoma* SEA and HCV core protein.

To confirm the *in vitro* results, 68 patients were enrolled (18 *Schistosoma* infected and 50 with chronic HCV and schistosomiasis coinfection) as well as 17 control healthy individuals. The *in vivo* data confirmed the *in vitro* data. There was a significant increase in the plasma levels of TNF-α and H_2O_2 in coinfected compared to those in *Schistosoma* infected patients (29.6% and 21.4%, respectively). Plasma TNF-α levels were not significantly ($p= 0.0953$) different between *Schistosoma* infected patients and controls (873.6 ± 269 pg/ml, and 536.5 ± 85.24 pg/ml, respectively). However, a significant 52% decrease in the level of plasma H_2O_2 was observed in *Schistosoma* infected patients compared to that in control subjects. It has been reported that H_2O_2 is involved in toxicity against *S. mansoni* [40].

Human blood plasma is continuously generating H_2O_2 [37], [38]. The increase in co-infected plasma H_2O_2 level in this study may be due to the increase in the level of xanthine oxidase which increases due to tissue injury and is usually involved in peroxide generation. This explanation is supported by previous studies [41]-[43].

In parallel to neutrophil mediators, the level of coinfected plasma IL-17 was markedly higher than that of *Schistosoma* infected patients or control subjects (80.6% and 42.6%, respectively). However, there was a non-significant decrease in plasma IL-17 levels from *Schistosoma* infected plasma compared to controls. Regarding, plasma IL-22 level in coinfected patients, it was markedly lower (51.3 %) compared to that from *Schistosoma* infected patients but not significantly different compared to that in control subjects. However, this IL-22 plasma level was significantly increased (43.6%) in *Schistosoma* infected plasma compared to that in control subjects.

The marked increase in IL-17 level in coinfected plasma is supported by several studies. Th17 cells have been shown to play an important pathogenic role in an increasing number of inflammatory [44]-[47] and infectious conditions [48], [49]. They regulate the immune responses by secreting IL-17 and thereby stimulating the production of additional proinflammatory and chemotactic molecules [50]. However, IL-22 which is also secreted by Th17 cells is reported to be a protective cytokine and so the decrease of its plasma level in coinfected patients comparing to *Schistosoma* infected ones is supported by this fact [11].

In conclusion, Th17 cytokines modulates neutrophil responses during co-infection with *Schistosoma* and HCV. Moreover, there are differential effects of IL-17 and IL-22 on neutrophil responses to co-infection with *Schistosoma* and HCV

ACKNOWLEDGMENT

The authors would like to thank the Science and Technology Development Fund (STDF) for supporting this study through the grant No. 1814 under US/Egypt cooperation program. Also, great thanks is paid to Dr Gamal Esmat, Professor of liver diseases, Department of Endemic Medicine and Hepatology, Faculty of Medicine, Cairo University for sending us the *Schistosoma* infected blood samples through our collaborative research project

TABLE I

DEMOGRAPHIC DATA OF THE ENROLLED SUBJECTS

***Schistosoma* patients (n= 17)**		**Co-infected patients (n= 50)**		**Controls (n= 17)**	
Sex	**Age ± SD**	**Sex**	**Age ± SD**	**Sex**	**Age ± SD**
Male (n= 14)	52.8 ± 11.5	Male (n= 20)	43.75 ± 7.4	Male (n= 7)	35 ± 16
Female (n= 3)	44.7 ± 4.7	Female (n= 30)	44.5 ± 8.4	Female (n= 10)	40 ± 13

FIGURE LEGENDS

Fig. 1: TNF-α production (A) and peroxide generation (B) by activated neutrophils in response to *Schistosoma* SEA/core-HCV.

Activation of neutrophils with SEA alone or core protein alone led to a significant decrease ($p< 0.001$) in the production of TNF-α by (17.39 ± 1.41 pg/ml) and (40.5 ± 17.45 pg/ml) respectively as compared to that of control non-activated neutrophils (100 ± 4.2 pg/ml). Additionally, there was a significant decrease in the production of TNF-α by neutrophils stimulated with both SEA and core protein (4.35 ± 1 pg/ml) as compared to SEA alone (17.39 ± 1.41 pg/ml) or core protein alone (40.5 ± 17.45 pg/ml) at $p < 0.001$ (Fig. 1A). However, hydrogen peroxide production was significantly increased by neutrophils stimulated with both SEA and core protein (142 ± 20 µMole/L) as compared to SEA alone (3 ± 0.15 µMole/L) or core protein alone (0 µMole/L) at $p < 0.001$ or controls as well (100 ± 2.25 µMole/L) at $p < 0.05$ (Fig. 1B). Although this production showed a significant decrease as compared to control in response to SEA alone or core protein alone.

Fig. 2: TNF-α production (A) and peroxide generation (B) by activated neutrophils in response to Schisto SEA/core-HCV in the presence of Th17 cytokines.

As shown in Fig. 2A&B, addition of IL-17 to SEA and core significantly increased TNF-α production (43.49 ± 2 pg/ml) but not hydrogen peroxide generation (120 ± 15 µMole/L) as compared to those without IL-17 by (4.35 ± 1 pg/ml) and (142 ± 20 µMole/L) respectively. However, addition of IL-22 significantly increased TNF-α (19.6 ± 7 pg/ml) and decreased hydrogen peroxide (38 ± 1.4µMole/L) as compared to those without IL-22 at $p < 0.001$ (Fig. 2A and B).

Fig. 3: Hydrogen peroxide and TNF-α levels in plasma of the enrolled subjects.

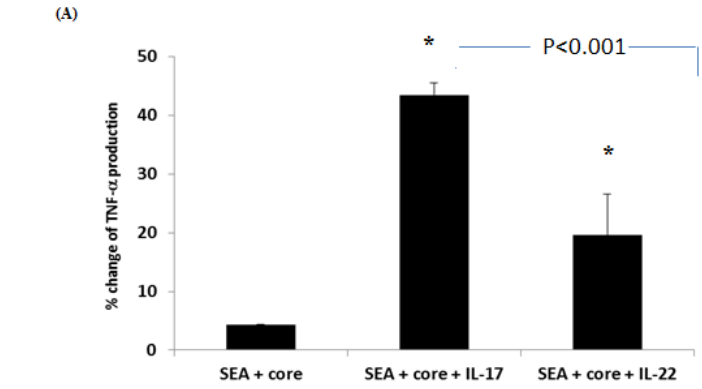
The *in vivo* results was confirmatory to the *in vitro* results where there was a significant increase in the level of TNF-α (1241.8 ± 232 pg/ml) and hydrogen peroxide (302.7 ± 48.8µMole/L) in coinfected plasma as compared to those in *Schistosoma* infected plasma (873.6 ± 269 pg/ml) and (237.7 ± 24.7µMole/L) respectively at $p < 0.05$ (Fig. 3).

There was a non-significant ($p= 0.0953$) increase in the level of TNF-α when Comparing *Schistosoma* infected plasma (873.6 ± 269 pg/ml) to control plasma (536.5 ± 85.24 pg/ml). However, a significant decrease in the level of peroxide was observed in *Schistosoma* infected plasma (237.7 ± 24.7µMole/L) as compared to that (494.7 ± 12.6 µMole/L) in control plasma at $p < 0.001$ (Fig. 3).

Fig. 4: Th17 cytokine levels in plasma of the enrolled subjects

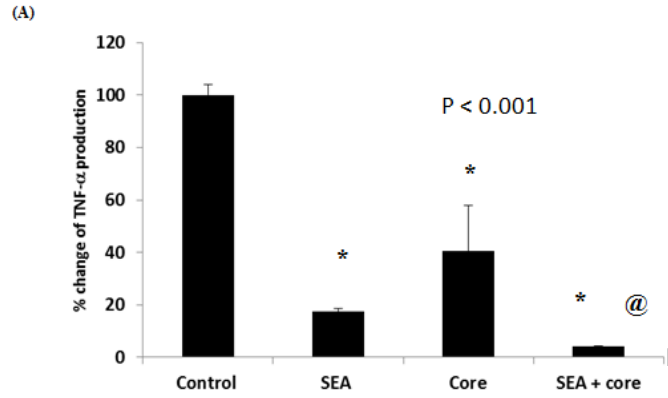
In parallel to neutrophil mediators, the level of coinfected plasma IL-17 was markedly higher (96.4 ± 12.5 pg/ml) than that in *Schistosoma* infected plasma (18.7 ± 4.8 pg/ml) or in control subjects (55.3 ± 27 pg/ml) at $p < 0.001$. However, there was a non-significant decrease in *Schistosoma* infected plasma as compared to controls ($p= 0.0817$) as shown in Fig. 4.

Regarding, IL-22 level it was markedly lower (0.54 ± 0.3 ng/ml) than that in *Schistosoma* infected plasma (1.11 ± 0.43 ng/ml) but not significant as compared to that in control subjects (0.625 ± 0.29 pg/ml) at p < 0. However, this level showed a significant increase in *Schistosoma* infected plasma (1.11 ± 0.43 ng/ml) as compared to that in control subjects at P< 0.05 01 (Fig. 4).

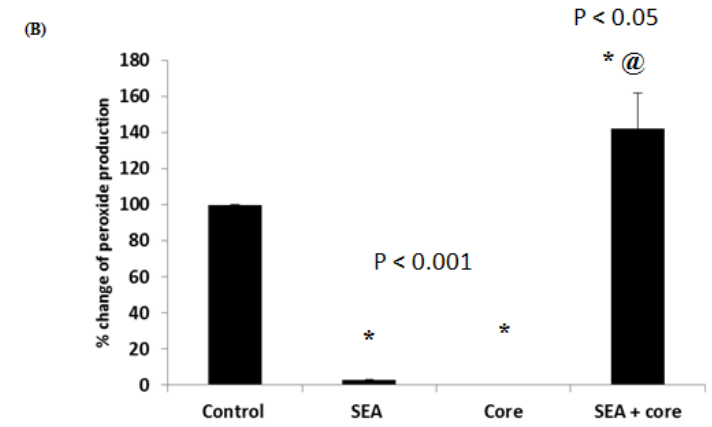


* Significantly different from controls at P< 0.05

Fig. 1: TNF-α production (A) and peroxide generation (B) by activated neutrophils in response to *Schistosoma* SEA/core-HCV.

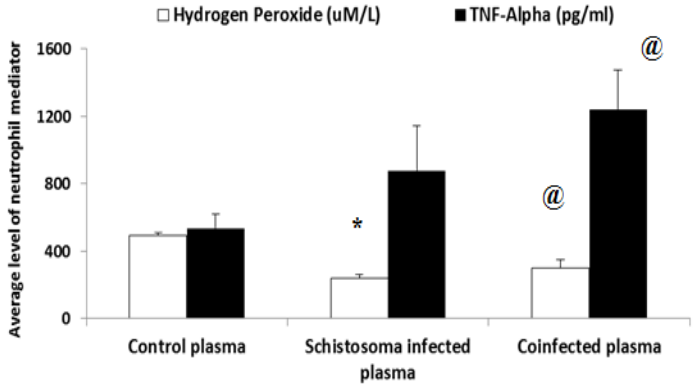


* Significantly different from controls at P< 0.001
@ Significantly different from SEA alone or Core alone at P< 0.001



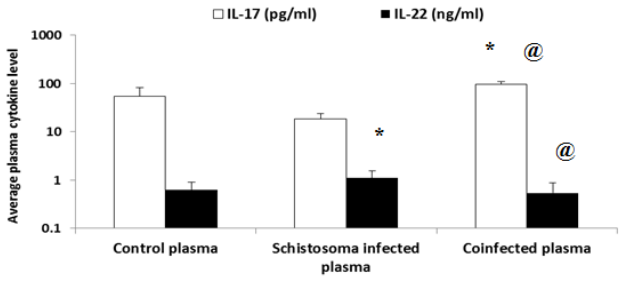
* Significantly different from controls at P< 0.001
@ Significantly different from SEA alone or Core alone at P< 0.05

Fig. 2: TNF-α production (A) and peroxide generation (B) by activated neutrophils in response to *Schistosoma* SEA/core-HCV in the presence of Th17 cytokines.



* Significantly different from controls at P< 0.05
@ Significantly different from SEA alone or Core alone at P< 0.05

Fig. 3: Hydrogen peroxide and TNF-α levels in plasma of the enrolled subjects.



* Significantly different from controls at P< 0.05
@ Significantly different from SEA alone or Core alone at P< 0.05

Fig. 4: Th17 cytokine levels in plasma of the enrolled subjects

REFERENCES

[1] Van-Lume, D.S., F. de Albuquerque Mde, A.I. de Souza, A.L. Domingues, E.P. Lopes, C.N. de Morais, and S.M. Montenegro," Association between Schistosomiasis mansoni and hepatitis C: systematic review", *Rev Saude Publica* Vol. 47, No. 2, 2013, pp. 414-424. http://dx.doi.org/10.1590/S0034-910.2013047004247

[2] Bassily, S., K.C. Hyams, N.A. el-Masry, N.F. Hassan, and D.M. Watts," Hepatitis C virus infection and hepatosplenic schistosomiasis", *Scandinavian journal of infectious diseases* Vol. 24, No. 5, 1992, pp. 687-688. http://dx.doi.org/10.3109/00365549209054660

[3] Kamel, M.A., F.D. Miller, A.G. el Masry, S. Zakaria, M. Khattab, G. Essmat, and Y.A. Ghaffar," The epidemiology of Schistosoma mansoni, hepatitis B and hepatitis C infection in Egypt", *Annals of tropical medicine and parasitology* Vol. 88, No. 5, 1994, pp. 501-509.

[4] Angelico, M., E. Renganathan, C. Gandin, M. Fathy, M.C. Profili, W. Refai, A. De Santis, A. Nagi, G. Amin, L. Capocaccia, F. Callea, M. Rapicetta, G. Badr, and G. Rocchi," Chronic liver disease in the Alexandria governorate, Egypt: contribution of schistosomiasis and hepatitis virus infections", *Journal of hepatology* Vol. 26, No. 2, 1997, pp. 236-243. http://dx.doi.org/10.1016/S0168-8278(97)80036-0

[5] Kamal, S.M., M.A. Madwar, T. Peters, R. Fawzy, and J. Rasenack,"Interferon therapy in patients with chronic hepatitis C and schistosomiasis", *Journal of hepatology* Vol. 32, No. 1, 2000, pp. 172-174. http://dx.doi.org/10.1016/S0168-8278(00)80207-X

[6] Strickland, G.T.," Liver disease in Egypt: hepatitis C superseded schistosomiasis as a result of iatrogenic and biological factors", *Hepatology* Vol. 43, No. 5, 2006, pp. 915-922. http://dx.doi.org/10.1002/hep.21173

[7] Frank, C., M.K. Mohamed, G.T. Strickland, D. Lavanchy, R.R. Arthur, L.S. Magder, T. El Khoby, Y. Abdel-Wahab, E.S. Aly Ohn, W. Anwar, and I. Sallam," The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt", *Lancet* Vol. 355, No. 9207, 2000, pp. 887-891. http://dx.doi.org/10.1016/S0140-6736(99)06527-7

[8] Mohamed-Ali, Q., N.E. Elwali, A.A. Abdelhameed, A. Mergani, S. Rahoud, K.E. Elagib, O.K. Saeed, L. Abel, M.M. Magzoub, and A.J. Dessein," Susceptibility to periportal (Symmers) fibrosis in human schistosoma mansoni infections: evidence that intensity and duration of infection, gender, and inherited factors are critical in disease progression", *J Infect Dis* Vol. 180, No. 4, 1999, pp. 1298-1306. http://dx.doi.org/10.1086/314999

[9] King, C.H., P. Magak, E.A. Salam, J.H. Ouma, H.C. Kariuki, R.E. Blanton, and O. World Health," Measuring morbidity in schistosomiasis mansoni: relationship between image pattern, portal vein diameter and portal branch thickness in large-scale surveys using new WHO coding guidelines for ultrasound in schistosomiasis", *Tropical medicine & international health : TM & IH* Vol. 8, No. 2, 2003, pp. 109-117. http://dx.doi.org/10.1046/j.1365-3156.2003.00994.x

[10] Kamal, S., M. Madwar, L. Bianchi, A.E. Tawil, R. Fawzy, T. Peters, and J.W. Rasenack," Clinical, virological and histopathological features: long-term follow-up in patients with chronic hepatitis C co-infected with S. mansoni", *Liver* Vol. 20, No. 4, 2000, pp. 281-289. http://dx.doi.org/10.1034/j.1600-0676.2000.020004281.x

[11] Eyerich, S., K. Eyerich, A. Cavani, and C. Schmidt-Weber," IL-17 and IL-22: siblings, not twins", *Trends Immunol* Vol. 31, No. 9, 2010, pp. 354-361. http://dx.doi.org/10.1016/j.it.2010.06.004

[12] Rutitzky, L.I., and M.J. Stadecker," Exacerbated egg-induced immunopathology in murine Schistosoma mansoni infection is primarily mediated by IL-17 and restrained by IFN-gamma", *Eur J Immunol* Vol. 41, No. 9, 2011, pp. 2677-2687. http://dx.doi.org/10.1002/eji.201041327

[13] Actor, J.K., M. Shirai, M.C. Kullberg, R.M. Buller, A. Sher, and J.A. Berzofsky," Helminth infection results in decreased virus-specific CD8+ cytotoxic T-cell and Th1 cytokine responses as well as delayed virus clearance", *Proceedings of the National Academy of Sciences of the United States of America* Vol. 90, No. 3, 1993, pp. 948-952. http://dx.doi.org/10.1073/pnas.90.3.948

[14] Kamal, S.M., L. Bianchi, A. Al Tawil, M. Koziel, K. El Sayed Khalifa, T. Peter, and J.W. Rasenack," Specific cellular immune response and cytokine patterns in patients coinfected with hepatitis C virus and Schistosoma mansoni", *The Journal of infectious diseases* Vol. 184, No. 8, 2001, pp. 972-982. http://dx.doi.org/10.1086/323352

[15] Rutitzky, L.I.," Severe CD4 T cell-mediated immunopathology in murine schistosomiasis is dependent on IL-12p40 and correlates with high levels of IL-17." *J Immunol* Vol. 175, 2005.

[16] Rutitzky, L.I.," *Mem Inst Oswaldo Cruz* Vol. 101, 2006.

[17] Rutitzky, L.I., P.M. Smith, and M.J. Stadecker," T-bet protects against exacerbation of schistosome egg-induced immunopathology by regulating Th17-mediated inflammation", *Eur J Immunol* Vol. 39, No. 9, 2009, pp. 2470-2481.

[18] Smith, P.M., M.G. Shainheit, L.E. Bazzone, L.I. Rutitzky, A. Poltorak, and M.J. Stadecker," Genetic control of severe egg-induced immunopathology and IL-17 production in murine schistosomiasis", *J Immunol* Vol. 183, No. 5, 2009, pp. 3317-3323.

[19] Ye, P., F.H. Rodriguez, S. Kanaly, K.L. Stocking, J. Schurr, P. Schwarzenberger, P. Oliver, W. Huang, P. Zhang, J. Zhang, J.E. Shellito, G.J. Bagby, S. Nelson, K. Charrier, J.J. Peschon, and J.K. Kolls," Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense", *J Exp Med* Vol. 194, No. 4, 2001, pp. 519-527.

[20] Huang, W., L. Na, P.L. Fidel, and P. Schwarzenberger," Requirement of interleukin-17A for systemic anti-Candida albicans host defense in mice", *J Infect Dis* Vol. 190, No. 3, 2004, pp. 624-631.

[21] Kelly, M.N., J.K. Kolls, K. Happel, J.D. Schwartzman, P. Schwarzenberger, C. Combe, M. Moretto, and I.A. Khan," Interleukin-17/interleukin-17 receptor-mediated signaling is important for generation of an optimal polymorphonuclear response against Toxoplasma gondii infection", *Infect Immun* Vol. 73, No. 1, 2005, pp. 617-621.

[22] Mollinedo, F., J.M. Nieto, and J.M. Andreu," Cytoplasmic microtubules in human neutrophil degranulation: reversible inhibition by the colchicine analogue 2-methoxy-5-(2',3',4'-trimethoxyphenyl)-2,4,6-cycloheptatrien-1- one", *Mol Pharmacol* Vol. 36, No. 4, 1989, pp. 547-555.

[23] Shata, M.T., A. Barrett, N.J. Shire, S.F. Abdelwahab, M. Sobhy, E. Daef, S.S. El-Kamary, M. Hashem, R.E. Engle, R.H. Purcell, S.U. Emerson, G.T. Strickland, and K.E. Sherman," Characterization of hepatitis E-specific cell-mediated immune response using IFN-gamma ELISPOT assay", *J Immunol Methods* Vol. 328, No. 1-2, 2007, pp. 152-161.

[24] Dambaeva, S.V., K.F. Kim, D.V. Mazurov, and V.I. Deigin," Effect of thymic peptides on the functional activity of phagocytic cells of donor peripheral blood", *Zh Mikrobiol Epidemiol Immunobiol*, No. 6, 2002, pp. 55-59.

[25] Pick, E., and Y. Keisari," A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture", *J Immunol Methods* Vol. 38, No. 1-2, 1980, pp. 161-170. http://dx.doi.org/10.1016/0022-1759(80)90340-3

[26] Gryseels, B., K. Polman, J. Clerinx, and L. Kestens," Human schistosomiasis", *Lancet* Vol. 368, No. 9541, 2006, pp. 1106-1118. http://dx.doi.org/10.1016/S0140-6736(06)69440-3

[27] Ghany, M.G., D.B. Strader, D.L. Thomas, and L.B. Seeff," Diagnosis, management, and treatment of hepatitis C: an update", *Hepatology* Vol. 49, No. 4, 2009, pp. 1335-1374. http://dx.doi.org/10.1002/hep.22759

[28] Abdel-Rahman, M., M. El-Sayed, M. El Raziky, A. Elsharkawy, W. El-Akel, H. Ghoneim, H. Khattab, and G. Esmat," Coinfection with hepatitis C virus and schistosomiasis: fibrosis and treatment response", *World J Gastroenterol* Vol. 19, No. 17, 2013, pp. 2691-2696. http://dx.doi.org/10.3748/wjg.v19.i17.2691

[29] Van-Lume, D.S., F. Albuquerque Mde, A.I. Souza, A.L. Domingues, E.P. Lopes, C.N. Morais, and S.M. Montenegro," Association between Schistosomiasis mansoni and hepatitis C: systematic review", *Rev Saude Publica* Vol. 47, No. 2, 2013, pp. 414-424. http://dx.doi.org/10.1590/S0034-910.2013047004247

[30] Wiedmann, M.W., J. Mossner, C. Baerwald, and M. Pierer," TNF alpha inhibition as treatment modality for certain rheumatologic and gastrointestinal diseases", *Endocr Metab Immune Disord Drug Targets* Vol.9,No.3,2009pp.295-314. http://dx.doi.org/10.2174/187153009789044347

[31] Tobin, A.M., and B. Kirby," TNF alpha inhibitors in the treatment of psoriasis and psoriatic arthritis", *BioDrugs* Vol. 19, No.1,2005, pp. 47-57. http://dx.doi.org/10.2165/00063030-200519010-00006

[32] Ho, S.C., K.S. Chang, and P.W. Chang," Inhibition of neuroinflammation by cinnamon and its main components", *Food Chem* Vol.138,No.4,2013,pp.2275-2282. http://dx.doi.org/10.1016/j.foodchem.2012.12.020

[33] Halliwell, B., and J.M. Gutteridge," Role of free radicals and catalytic metal ions in human disease: an overview", *Methods Enzymol* Vol. 186, 1990, pp. 1-85. http://dx.doi.org/10.1016/0076-6879(90)86093-B

[34] Evans,P.,and B.Halliwell,"Free radicals andhearing.Cause, consequence, and criteria", *Ann N Y Acad Sci* Vol. 884, 1999, pp.19-40. http://dx.doi.org/10.1111/j.1749-6632.1999.tb08633.x

[35] Halliwell, B.," Antioxidant defence mechanisms: from the beginning to the end (of the beginning)", *Free Radic Res* Vol. 31, No. 4, 1999, pp. 261-272. http://dx.doi.org/10.1080/10715769900300841

[36] Long, L.H., A.N. Lan, F.T. Hsuan, and B. Halliwell," Generation of hydrogen peroxide by "antioxidant" beverages and the effect of milk addition. Is cocoa the best beverage?" *Free Radic Res* Vol. 31, No. 1, 1999, pp. 67-71.

[37] Halliwell, B., M.V. Clement, and L.H. Long," Hydrogen peroxide in the human body", *FEBS Lett* Vol. 486, No. 1, 2000, pp. 10-13.

[38] Halliwell, B., M.V. Clement, J. Ramalingam, and L.H. Long," Hydrogen peroxide. Ubiquitous in cell culture and in vivo?" *IUBMB Life* Vol. 50, No. 4-5, 2000, pp. 251-257.

[39] Kolls, J.K., and A. Linden," Interleukin-17 family members and inflammation", *Immunity* Vol. 21, No. 4, 2004, pp. 467-476.

[40] Hahn, U.K., R.C. Bender, and C.J. Bayne," Involvement of nitric oxide in killing of Schistosoma mansoni sporocysts by hemocytes from resistant Biomphalaria glabrata", *J Parasitol* Vol. 87, No. 4, 2001, pp. 778-785.

[41] Friedl, H.P., D.J. Smith, G.O. Till, P.D. Thomson, D.S. Louis, and P.A. Ward," Ischemia-reperfusion in humans. Appearance of xanthine oxidase activity", *Am J Pathol* Vol. 136, No. 3, 1990, pp. 491-495.

[42] Lacy, F., D.A. Gough, and G.W. Schmid-Schonbein," Role of xanthine oxidase in hydrogen peroxide production", *Free Radic Biol Med* Vol. 25, No. 6, 1998, pp. 720-727. http://dx.doi.org/10.1016/S0891-5849(98)00154-3

[43] Houston, M., A. Estevez, P. Chumley, M. Aslan, S. Marklund, D.A. Parks, and B.A. Freeman," Binding of xanthine oxidase to vascular endothelium. Kinetic characterization and oxidative impairment of nitric oxide-dependent signaling", *J Biol Chem* Vol. 274, No. 8, 1999, pp.4985-4994. http://dx.doi.org/10.1074/jbc.274.8.4985

[44] Afzali, B., G. Lombardi, R.I. Lechler, and G.M. Lord," The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease", *Clin Exp Immunol* Vol. 148, No.1, 2007, pp. 32-46. http://dx.doi.org/10.1111/j.1365-2249.2007.03356.x

[45] Baumgart, D.C., and S.R. Carding," Inflammatory bowel disease: cause and immunobiology",*Lancet* Vol. 369, No. 9573, 2007, pp. 1627-1640. http://dx.doi.org/10.1016/S0140-6736(07)60750-8

[46] Paradowska, A., W. Masliniski, A. Grzybowska-Kowalczyk, and J. Lacki," The function of interleukin 17 in the pathogenesis of rheumatoid arthritis", *Arch Immunol Ther Exp (Warsz)* Vol. 55, No. 5, 2007, pp. 329-334. http://dx.doi.org/10.1007/s00005-007-0032-8

[47] Schmidt-Weber, C.B. M. Akdis, and C.A. Akdis," TH17 cells in the big picture of immunology", *J Allergy Clin Immunol* Vol. 120,No.2,2007, pp.247-254. http://dx.doi.org/10.1016/j.jaci.2007.06.039

[48] Bettelli, E., M. Oukka, and V.K. Kuchroo," T(H)-17 cells in the circle of immunity and autoimmunity", *Nat Immunol* Vol. 8, No. 4, 2007, pp. 345-350. http://dx.doi.org/10.1038/ni0407-345

[49] Weaver, C.T., R.D. Hatton, P.R. Mangan, and L.E. Harrington," IL-17 family cytokines and the expanding diversity of effector T cell lineages", *AnnuRevImmunol*Vol.25,2007,pp.821-852. http://dx.doi.org/10.1146/annurev.immunol.25.022106.141557

[50] Romagnani, S.," Regulation of the T cell response", *Clin Exp Allergy* Vol. 36, No. 11, 2006, pp. 1357-1366. http://dx.doi.org/10.1111/j.1365-2222.2006.02606.x