

The role of interleukin-2 and interleukin-4 in disease progression in patients with schistosomiasis combined with chronic viral hepatitis C and chronic viral hepatitis B

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Summary

Background: Schistosomiasis remains an important cause of parasitic morbidity and mortality worldwide. In countries where schistosomiasis is endemic, a high prevalence of combined Schistosomiasis mansoni and chronic hepatitis C and chronic hepatitis B co-infection has been described. The immunopathogenesis of human schistosomiasis with or without chronic hepatitis remains controversial. **Aim of the work:** Is to evaluate the immunopathogenic role of Th1 cytokine (IL-2) and Th2 cytokine (IL-4) in patients with different stages of hepatointestinal schistosomiasis with or without chronic hepatitis. **Methods:** The study included 60 Egyptian patients with different stages of hepatointestinal schistosomiasis. They include 40 patients with hepatosplenic schistosomiasis (divided into 4 subgroups according to stage of hepatosplenic schistosomiasis), 10 patients with schistosoma mansoni and chronic hepatitis C and 10 patients with schistosoma mansoni and chronic hepatitis B co-infection, In addition to, 10 healthy controls. IL-2 and IL-4 was measured by competitive enzyme immunoassay (EIA) which measures the natural and recombinant forms of the cytokines. **Results:** Compared to control group, patients with hepatosplenic schistosomiasis showed significant increase in serum IL-2 from stage 1 ($P < 0.05$) to stage 4 ($P < 0.001$). Moreover, both groups with combined schistosomiasis and chronic hepatitis C and chronic hepatitis B showed significant increase in the IL-2 ($P < 0.05$ for both). Furthermore, decompensated schistosomiasis showed highly significant increase in the IL-2 versus compensated schistosomiasis ($P < 0.001$). Also, compared to control group, patients with hepatosplenic schistosomiasis showed significant increase in serum IL-4, that decreased from stage 1 ($P < 0.001$) to non-significant increase in stage 4 ($P > 0.05$). In addition, chronic HCV with schistosomiasis showed significant increase in the IL-4 ($P < 0.05$) and chronic HBV with schistosomiasis showing significant increase in the IL-4 ($P < 0.001$). Moreover, compensated schistosomiasis showed significant increase in the IL-4 ($P < 0.001$) versus decompensated schistosomiasis. **Conclusion:** Patients with chronic schistosomiasis present a mixed profile of Th1 and Th2 cytokines. The Th1 (IL-2) was increased with the progression of the hepatosplenic schistosomiasis from stage 1 (hepatomegaly) to the stage 4. However, The Th2 (IL-4) was elevated in the first stage of hepatosplenic schistosomiasis and then decreased with progression of the disease. In patients with combined schistosomiasis and chronic hepatitis the Th2 (IL-4) is the predominant cytokine versus Th1 (IL-2).

Keywords: Schistosomiasis, chronic hepatitis, interleukin-2 and interleukin-4

Introduction

Human schistosomiasis is one of the most harmful parasitic diseases. It is reported to be endemic in 77 countries in tropical and subtropical regions, leading to infection of about 250 million individuals universal¹. Schistos-

omiasis is a disease caused predominantly by the host's immune response to schistosoma ova and the granulomatous reaction they induce¹⁻⁶. The granulomas destroy the eggs and sequester or neutralize otherwise pathogenic egg antigens

Medical Journal of Viral Hepatitis

(MJVH) 2019; 3 (2) - pp. 57-64

Received: 18/12/2018

Revised: 8/1/2019

Accepted: 20/1/2019

Published Online: 25/4/2019

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but also leads to fibrogenesis in host tissues⁶. An effective T-cell response is known to be essential for the development of the granulomatous response and host survival⁷. Studies in human patients of the association between disease severity and the production of cytokines have shown that, different clinical forms of schistosomiasis are associated with distinct immunological profiles^{8,9}. Moreover, studies proposed that, the outcome of human schistosomiasis is influenced by the nature of the Th1/Th2 immune response against schistosome antigens¹⁰. Yet, there is no strong consensus about the pattern of cytokine production and regulation that causes severe hepatosplenic (HS) disease, which is characterized by periportal fibrosis and portal hypertension. HS disease and the progression of hepatic fibrosis are associated with the production of profibrotic type 2 cytokines in the early stages of infection with schistosoma mansoni. But, other studies demonstrated that, HS disease is characterized by a predominant T helper 1 profile¹¹. Concurrent infection between hepatitis B virus (HBV) and schistosomiasis is well known in countries where schistosomiasis is endemic and might cause chronic liver inflammation¹². S. mansoni and HBV co-infection pathogenesis remains debated; however, the harmful effects of S. mansoni and hepatitis C virus (HCV) co-infection on liver fibrosis have confirmed in endemic countries¹³⁻¹⁵. In countries where schistosomiasis is endemic for example Egypt, a high prevalence of HBV and S. mansoni co-infection has been described ranging from 19.6 to 33.0%^{16,17}. Similarly, published data about HCV and schistosomiasis co infection is scanty. Prevalence rates of HCV infection with wide variations as low as 1% and as high as 50% among patients with schistosomiasis, were reported in many countries^{18,19}. Likewise high (40.2%) prevalence of HCV and S. mansoni antibodies were in some study²⁰. Interestingly, a study among 3,596 Egyptian patients found that 27.3% had both HCV-RNA and schistosomiasis, though a weak point is their reliance on serology to diagnose schistosomiasis, a thing that questions the association between HCV and schistosomiasis²¹. Our aim is to study the impact of IL-2 and IL-4 in-patient with chronic schistosomiasis and hepatitis B or C co-infection.

Subjects and Methods

Patients

This study was carried out on 70 Egyptian cases attending clinics of Tropical Medicine and

Internal Medicine Departments (inpatient and outpatient) Mansoura university Hospital. They include 40 patients with hepatosplenic schistosomiasis (20 males and 20 females), 10 patients with chronic HCV infection (8 males and 2 females) and 10 patients with chronic HBV infection (6 males and 4 females). In addition to, 10 healthy control (5 males and 5 females), mean age in years was (37.30 ±15.20). The studied cases were classified into 4 groups:

Group 1: Cases of hepatosplenic schistosomiasis include 40 patients who are further classified into four subgroups according to stages of hepatosplenic schistosomiasis²². **Group 1-A:** Hepatosplenic schistosomiasis stage 1 (Hepatomegaly), include 10 patients (4 males and 6 females) with mean age in years (39.10 ±13.12). **Group 1-B:** Hepatosplenic schistosomiasis stage 2 (Hepatosplenomegaly) include 10 patients (3 males and 7 females) with mean age in years (40.20 ±12.91). **Group 1-C:** Hepatosplenic schistosomiasis stage 3 (splenomegaly with shrunken liver), include 10 patients (7 males and 3 females) with mean age in years (38.20 ±10.71). **Group 1-D:** Hepatosplenic schistosomiasis stage 4 (Decompensated schistosomiasis), include 10 patients (6 males and 4 females) with mean age in years (41.80 ±9.86). Patients with schistosomiasis were also classified again according to clinical stage into: **Compensated schistosomiasis:** (without ascites), include 30 patients (14 males and 16 females) with mean age (39.15 ±12.58). **Decompensated schistosomiasis:** (with ascites) include 10 patients (6 males and 4 females) with mean age in years (41.80 ± 9.86).

Group 2: Cases of schistosomiasis with chronic hepatitis C virus include 10 patients (8 males and 2 females) with mean age in years (41.80 ±18.1095).

Group 3: Cases of schistosomiasis with chronic hepatitis B virus include 10 patients (6 males and 4 females) with mean age in years (42.20 ± 8.1486). Control group, include 10 patients (5 males and 5 females) with mean age in years (37.30 ±15.2032).

Group 4: 10 healthy control (5 males and 5 females), mean age in years was (37.30 ±15.20)

Exclusion criteria

Autoimmune diseases, diabetes mellitus, malignancies, hematological diseases, allergic skin diseases, connective tissue or collagen diseases, cardiovascular diseases, kidney diseases, bac-

terial or fungal diseases, and other parasitic diseases. All patients and control groups were subjected to the following; thorough history taking, clinical examination and abdominal ultrasonography. The study was carried out in accordance with the guidelines of the Helsinki Declaration.

Laboratory investigations

Included; liver functions (transaminases serum bilirubin, albumin, prothrombin time), serum creatinine, complete blood picture and stool and urine examination. Viral markers (HAV, HBV, HCV and HIV) were screened by ELISA technique- Kits were provided by Abbott Diagnostic). Stool culture, rectal snip biopsies were done. Interlukin-2 and Interlukin-4 were measured by ACCUCYTE human IL-2 and IL-4 (cytimmune Sciences Inc.), a competitive enzyme immunoassay (EIA), which measures the natural and recombinant forms of the cytokines IL-2 and IL-4.

Statistical analysis

Statistical analysis was done by SPSS (statistical package for social science) program version 10. The qualitative data were presented in the form of number and percentage. Chi-square test was used as a test of significance for qualitative data. The quantitative data were presented in the form of mean, standard deviation. Student t-test was used as a test of significance for quantitative data.

Results

This study was carried out on 70 Egyptian patients. They include 40 patients with hepatosplenic schistosomiasis with mean age in years (39.90 ± 13.12), 10 patients with chronic HCV infection with mean age in years (41.80 ± 18.10) and 10 patients with chronic HBV infection with mean age in years (42.20 ± 8.14). In addition to, 10 healthy control with mean age in years was (37.30 ± 15.20), tab. (1). Table (2) shows the laboratory data of the studied groups. As regarding the serum albumin, patients with hepatosplenic schistosomiasis showed gradual decreasing from stage 1 where was the mean value (4.01 g/dl) down to the stage 4 (decompensated schistosomiasis) where was the mean value (2.27 g/dl) ($p = P < 0.001$). In both groups of schistosomiasis with chronic hepatitis there was, decrease in serum albumin, where the mean value in chronic hepatitis C with schistosomiasis was (2.36 g/dl) and in

chronic hepatitis B was (2.14 g/dl) ($p = P < 0.001$ for both groups). As regard the serum bilirubin, all hepatosplenic schistosomiasis showing non-significant increase. However, in both groups of schistosomiasis with chronic hepatitis there was significant increase in both groups where, the mean value in chronic hepatitis C with schistosomiasis was (1.6 mg/dl) and in chronic hepatitis B was (1.7 mg/dl) ($P < 0.001$ for both groups). As regard the ALT and AST levels, all hepatosplenic schistosomiasis showed non-significant increase. However, in both groups of schistosomiasis with chronic hepatitis there was significant increase in both groups where the mean value was in chronic hepatitis C with schistosomiasis (61.2 IU/l) and in chronic hepatitis B (52.2 IU/L). Concerning AST levels in both groups of schistosomiasis with chronic hepatitis, there was significant increase in both groups where the mean value was in chronic hepatitis C with schistosomiasis (60.8 IU/l) and in chronic hepatitis B (55.8 IU/l). Table (3) shows the significance of the IL-2 (Th1) in the studied groups versus the control group. Compared to control group, patients with hepatosplenic schistosomiasis showed significant increase in serum IL-2, stage 1 ($P < 0.05$), stage 2 ($P < 0.01$), stage 3 ($P < 0.01$) and stage 4 ($P < 0.001$). Moreover, Chronic HCV with schistosomiasis showed a significant increase in the IL-2 ($P < 0.05$) and chronic HBV with schistosomiasis showed significant increase in the IL-2 ($P < 0.05$). Table (4) shows the significance of the IL-4 (Th2) in the studied groups versus the control group. Compared to control group, patients with hepatosplenic schistosomiasis showed significant increase in serum IL-4, stage 1 ($P < 0.001$)), stage 2 ($P < 0.001$)), stage 3 ($P < 0.01$) while, hepatosplenic schistosomiasis stage 4 showing non-significant increase in the IL-4 ($P > 0.05$). Furthermore, chronic HCV with schistosomiasis showed significant increase in the IL-4 ($P < 0.05$) and chronic HBV with schistosomiasis showing significant increase in the IL-4 ($P < 0.001$). Furthermore, decompensated schistosomiasis showed highly significant increase in the IL-2 versus compensated schistosomiasis ($P < 0.001$) fig. (1). However, compensated schistosomiasis showed significant increase in the IL-4 versus decompensated schistosomiasis ($P < 0.001$) fig. (2).

Table (1) The age and sex distribution of studied groups

Group	Hepatosplenic schistosomiasis N=40	Chronic HCV and schistosomiasis N= 10	Chronic HBV and schistosomiasis N=10	Control N=10
Age/ year	39.90±13.12	41.80±18.10	42.20±8.14	37.30±15.20
Gender: male/female	20/20	8/2	6/4	5/5

P=0.069 for age distribution, and P= 0.169 for sex distribution

Table (2) Laboratory data of studied groups

Stages of Hepatosplenomegaly	Bilirubin Mean ± SD	ALT Mean±SD	AST Mean±SD	Albumin Mean±SD
Hepatosplenomegaly stage 1	0.831±0.10	29.71±2.60	35.20±2.41	4.01±0.21
Hepatosplenomegaly stage 2	0.86±0.10	35.21±3.12	33.120±3.32	3.95±0.12
Hepatosplenomegaly stage 3	0.87±0.10	36.12±3.21	34.21±4.99	3.93±0.41
Hepatosplenomegaly stage 4	1.00±0.17	33.70 ±3.56	32.20±4.09	2.27±0.22
Chronic HCV with schistosomiasis	1.69±0.45	61.20±9.30	60.80±12.34	2.36±0.29
Chronic HBV with schistosomiasis	1.75±0.27	52.20±4.93	55.80±10.68	2.14±0.10

Table (3): IL-2 ng/ml of the studied groups

Groups	Mean± SD	P value
Hepatosplenic schistosomiasis: stage 1	190.23±20.91	< 0.05
Hepatosplenic schistosomiasis: stage 2	230.12±50.12	0.01
Hepatosplenic schistosomiasis: stage 3	310.91±96.17	0.01
Hepatosplenic schistosomiasis: stage 4	367.1±12.91	0.001
Chronic HCV and schistosomiasis	255±16.91.0	< 0.05
Chronic HBV and schistosomiasis	267.15±25.12	< 0.05
Control group	167.6± 70.67	

P value versus control group

Table (4): IL-4 ng/ml of the studied groups

Groups	Mean±SD	P value
Hepatosplenic schistosomiasis: stage 1	360.12±60.12	P< 0.001
Hepatosplenic schistosomiasis: stage 2	340.17±70.14	P< 0.001
Hepatosplenic schistosomiasis: stage 3	287.12±69.12	P< 0.01
Hepatosplenic schistosomiasis: stage 4	250.1±99.1	P> 0.05
Chronic HCV and schistosomiasis	467.4±109.9	P< 0.05
Chronic HBV and schistosomiasis	436.1±63.18	P< 0.001
Control	202.1±42.21	

P value versus control group

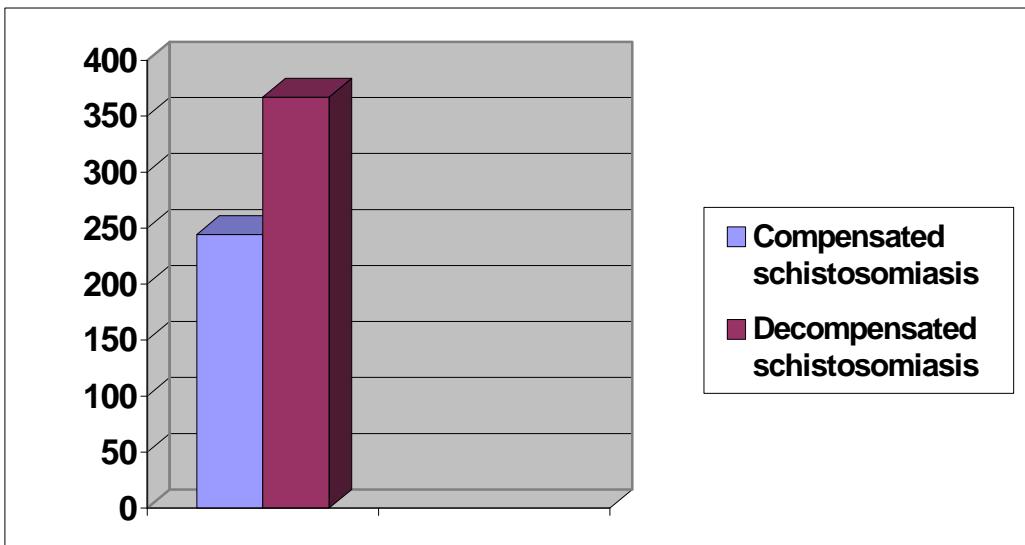


Figure (1) IL-2 in compensated and decompensated schistosomiasis.

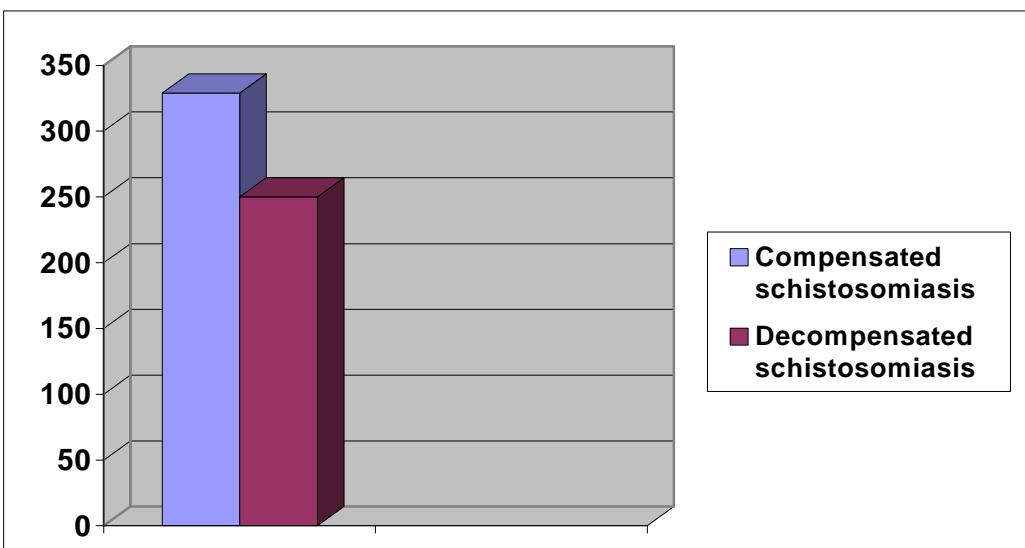


Figure (2): IL-4 in compensated and decompensated schistosomiasis.

Discussion

Our study demonstrated that, the IL-4 (Th2) was extremely significantly high in stage I where was the ($P < 0.001$) and then decreases progressively to stage 3, where was ($p < 0.01$) then, to non-significant increase in stage 4 ($P > 0.05$). We could explain these changes in cytokines pattern in this group of patients by that, the Th2 (IL-4) is responsible for the initiating of the schistosomal pathology and granuloma formation, and the Th1 (IL-2) is associated with the progression of the disease. These results were in agreement with Fallon et al, who demonstrated that, IL-4 drives the development of the granulomatous response whereas IL-13 is the key profibrotic cytokine in

the development of schistosoma induced hepatic fibrosis²³. Moreover, IL-4 is responsible for determining granuloma size, inducing the proliferation of Th2 cytokine-producing lymphocytes^{24,25}. Also, De Morais et al, found that, patients with intestinal schistosomiasis typically display a mixed Th1/Th2 response with higher levels of IL-4 production in comparison to acute schistosomiasis⁸. Additionally, high levels of the Th2 cytokines IL-4 correlate with the persistence of fibrosis following treatment with praziquantel²⁶. Overall, the results of these studies tend to corroborate those in mice suggesting that Th2 cytokines, including IL-4 and IL-13, promote immunop-

atology¹⁰. In our study we found that, the level of IL-2 (Th1) was increased progressively from stage 1 (hepatomegaly) where was ($P < 0.05$) to stage 4 (huge splenomegaly with shrunken liver) where was the ($p < 0.001$). In accordance with our results, previous studies demonstrated that, the dynamics of IL-2 production by splenic cells of schistosoma mansoni infected mice was correlated with the intensity of hepatic granulomatous inflammation²⁷. This explains the progressive increasing of IL-2 from stage 1 to stage 4 of hepatic granulomatous inflammation. To study the role of the Th1 and Th2 as regarding the activity and severity of the disease, hepatosplenic schistosomiasis both compensated (without ascites) and decompensated (with ascites) groups were assayed for IL-2 (Th1) and IL-4 (Th2). The results were, extremely significant increase ($P < 0.001$) in decompensated group versus compensated group as regarding (IL-2), but significant increase in compensated versus decompensated group ($P < 0.001$) as regarding (IL4. This means that Th1 is responsible for progression and more severe form of the hepatosplenitis schistosomiasis. These results were in agreement with, Mwatha et al, who showed that, in both mice and humans infected with schistosoma mansoni, diminished type 2 and heightened type I responses are associated with severe morbidity²⁸. In addition, Fallon et al, showed that, more convincingly evidence from schistosoma infected humans suggest that, proinflammatory type I responses are the cause morbidity in schistosomiasis²⁹. Moreover, data indicated that more severe form of schistosoma infection (hepatosplenomegaly) is associated with Th1 profile following in vitro stimulation with antigen²⁸. However, the our results are in contrast to the data that demonstrated that, in context of the type1/type2 cytokines paradigm, data from mice and humans have categorized schistosomiasis as type 2 disease, implicating type2 responses as the cause of morbity and being detrimental to the host³⁰. Moreover, decreased fibrosis was associated with diminished Th2 –responses and accentuated type1 cytokines production. Additionally, mAb anti IL-4 treatment of mice tends to reduce granuloma size and fibrosis³¹. In this study it was reported that patients, with schistosomiasis and viral hepatitis C showing highly significant increase in IL-4 (Th2) ($P < 0.001$) and significant increase in IL-2 ($P < 0.05$) when compared with control group. Patients

with schistosomiasis and chronic hepatitis B showing highly significant increase in IL-4 (Th2) ($P < 0.001$) and significant increase in IL-2 ($P < 0.05$) when compared with control group. This means that, in co-infected patients with schistosomiasis and viral hepatitis (chronic HCV and HBV), the Th2 was prominent than Th1. These results were in agreement with Kamal et al., who showed that, co-infected patients with schistosomiasis and viral hepatitis had high IL-4 and IL-10 (Th2 profile), whereas patients infected with chronic hepatitis alone had higher IFN- γ and TNF- α (Th1 profile)³². It was showed that, in combined schistosomiasis and chronic hepatitis, Th2 profile (IL-4) induced by schistosomiasis antagonized and down regulated the antiviral activities of Th1 cytokines, resulting in increased viral replication and more progression to fibrosis. Imbalance toward a Th2 profile at the time of acquisition of viral infection or during the acute stage of the disease may favor the faster progression to chronicity in co-infected patients^{33,34}. In contrast to results of this study is that, Egyptian patients infected with HCV genotype 4 can mount HCV- specific T cell responses, both CD4+ and CD8+ T cell-mediated, despite the prevalence of concomitant schistosomiasis³⁵.

Conclusion

Patients with chronic schistosomiasis present a mixed profile of Th1 and Th2 cytokines. The Th2 (IL-4) was elevated in the first stage of hepatosplenitis schistosomiasis and then decreased with progression of the disease while, the Th1 (IL-2) was increased with the progression of the hepatosplenitis schistosomiasis from stage 1(hepatomegaly) to the stage 4 (decompensated schistosomiasis). In patients with combined schistosomiasis and chronic hepatitis the Th2 (IL-4) is the predominant cytokine versus Th1 (IL-2).

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