Review Article

Interaction Between the Neglected Tropical Disease Human Schistosomiasis and HCV Infection in Egypt: a Puzzling Relationship

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Abstract

Egypt has the highest prevalence of chronic hepatitis C virus (HCV) infection and seropositivity worldwide, and it has been proposed that this enhanced susceptibility to HCV is related to coinfection with schistosomiasis. Although currently, there are no studies regarding the actual prevalence of both human schistosomiasis and schistosomiasis/HCV coinfection evidences strongly support that eliminating human schistosomiasis from Egypt is necessary to reduce both HCV prevalence and liver pathology. The present review highlights the significant impact of the neglected tropical disease human schistosomiasis on both susceptibility of Egyptians to HCV coinfection, severity of the resulting liver pathology, and poor response to antiviral therapy. The immune evasion mechanisms exerted by the HCV-NS3/4A protease domain, and the possible impact of immune evasion mechanisms exerted by proteases of larval, worm and egg stages of the parasite *Schistosoma* on human susceptibility to HCV infection are discussed. In addition, schistosome immune evasion mechanisms may include immunosuppression that in turn prevents clearance of HCV viremia and leads to relapsing HCV infection and severe liver pathology. I propose the generation of a replicon system from the most prevailing genotype (HCV-4a) in Egypt and establishing its replication on hepatoplastoma or immune cells in presence of bilharzial antigens. Finally, the use of a humanized small animal model that can acquire both HCV and *S. mansoni* infections will be important to further understand in real time the impact of coinfection on both the immune system and liver pathology.

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Keywords: Egypt; Neglected tropical disease; Human schistosomiasis; HCV; Coinfection; Immune evasion mechanisms; HCV protease; Schistosome proteases.

Abbreviations: HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HCV-4a, HCV genotype 4a; INF- γ, interferon gamma; IPS-1, interferon-beta promoter stimulator 1; IRF-3, IFN regulatory factor 3; ISGs, IFN-stimulated genes; MAVS, mitochondrial anti-viral signaling protein; NS, nonstructural protein; RIG-I, retinoic-acid inducible gene I; TNF- α, tumor necrosis factor alpha; TLR3, Toll-like receptor 3; VISA, virus-induced signaling adaptor.

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Introduction

Helminth infections are one of the most common parasitic diseases found in tropical countries worldwide.[1] The highest prevalence of such infections occur in underdeveloped regions with poor hygiene and inadequate water supply and sanitation. These populations often live on less than 2 US dollars per day. Helminthic infections are the most neglected of tropical diseases.[1] Schistosomiasis, in particular, has been endemic in Egypt and other African countries located along the Nile River since antiquity.[2] During the first half of the 20th century, up to 80% of the residents in impoverished Egyptian villages were infected with schistosome parasites, an incident that made a writer at that time declare, "No other people on earth suffer the consequences of bilharzial infection to the extent of the Egyptian farmers".[2] Risk factors for active schistosomiasis transmission, including improper water and sanitation facilities and bodies of stagnant water contaminated with infected intermediate snail hosts, existed in poor Egyptian villages and dramatically contributed to the establishment of disease endemicity.[2] Globally, chronic morbidities associated with repeated schistosomiasis infection include impaired child growth and development, chronic liver inflammation, anemia, and other nutritional deficiencies. It has been estimated that up to 56 million disability adjusted life years are lost annually, a value that exceeds the estimated global burden of malaria.[3]

Prior to the availability of oral praziquantel, the primary treatment for schistosomiasis in Egypt was tartar emetic (potassium antimony tartrate) administered parenterally.[2] Hundreds of thousands of Egyptian farmers received the medication following World War I, which was, although highly toxic, often effective in curing schistosomiasis. The drug exerted its action via inhibition of DNA synthesis,[4] and side effects included nausea, vomiting, coughing, acute circulatory failure, and electrocardiographic changes.[5] Unfortunately, this drug was dispensed without due attention to sterilization of needles between patients and resulted in an unanticipated epidemic of hepatitis C virus (HCV) transmission.[6–12] During this period, human blood and multiple syringe use were

not known as possible routes for transmission of other infections.[12] It has recently become evident that the parenteral administration of tartar emetic using non sterilized syringes resulted in widespread transmission of both HCV and HBV.[13] Given the prevalence of schistosomiasis/HCV coinfection in Egyptians, higher susceptibility of this population was proposed.[14,15] Two years ago, the prevalence of chronic HCV infections and seropositivity among Egyptians was reported to be one in 10 people, whereas the global rate was one in 50 people.[16] In contrast, the seroprevalence of HCV and schistosomiasis in neighboring Sudan is much lower, and the reason for this difference is still unclear.[17]

Over the last decade, most papers published regarding human schistosomiasis began with a similar version of the following paragraph: "Schistosomiasis is the second most important parasitic infection after malaria and affects more than 200 million people in 74 countries.[18] It is endemic, with high prevalence and morbidity rates in many countries, especially those in Africa, such as Egypt,[19] Kenya[20] and Sudan[12,22] and in South America, mainly Brazil.[23] The largest epidemiological survey in Egypt mentioned the prevalence of *Schistosoma haematobium* in Upper Egypt (where it is endemic) to be around 7.8%, while the prevalence of S. mansoni in Lower Egypt (where it is endemic) was found to be around 36.4%.[19] When one carefully looks at the dates of the references for global prevalence of the human schistosomiasis, it becomes apparent that all results are outdated and represent more a history than a description of the current status.

The main reasons for this is that human schistosomiasis, among other helminth infections, became an extremely neglected tropical disease.[1] During the 1990's, the WHO, the Egyptian Government in collaboration with the USAID,[24] and the EU allocated large funds to establish relationships between scientists from endemic countries for human schistosomiasis and collaborators form Europe and the USA. These funds were extremely useful in training young scientists and establishing an infrastructure in Egypt that enabled laboratories to seriously begin research on the control of human schistosomiasis. Unfortunately, the majority of the funds was spent on characterizing vaccine candidates, an aim which is to date still not achieved, and not on setting up concrete hygienic plans to eliminate human schistosomiasis from Egypt. Candidate schistosome antigens that were evaluated for their protective potential against schistosomiasis infection and their capacity to stimulate various host immune responses are listed in table 1 (reviewed in [25]).[26–49] By the end of the 1990's, the funds expired, Western countries excluded human schistosomiasis from their research priorities, and Egypt declared schistosomiasis as a minor or no longer existing health problem. The dramatic absence of both international funding schemes and political willingness to help contributed to an extreme neglect of human schistosomiasis and other helminth, soil-, and mosquitoes-borne diseases in Egypt over the last twenty years.

The early schistosomiasis tartar emetic control campaign which resulted in HCV transmission[8–13] led to the highest prevalence rates globally of HCV in Egypt,[17] with many cases developing into chronic liver cirrhosis and hepatocellular carcinoma (HCC). *Schistosoma* and HCV appear to act synergistically in coinfected patients, causing more severe liver disease progression compared to either pathogen alone.[50–60] Since praziquantel became the oral drug of choice to control human schistosomiasis,[61] no injectable drugs are used for infection treatment. Nevertheless, HCV infection is frequently complicated by underlying *S. mansoni* coinfection, which has been associated with increased HCV morbidity and chronicity.[62–64]

Taken together, these findings make clear that although schistosomiasis is an extremely neglected tropical disease, it has contributed significantly to the spread of, the susceptibility to, and the pathological consequences of HCV infection.

The present review focuses on the immune evasion mechanisms of both schistosome parasites and HCV that might underlie the higher susceptibility of, and the severe disease outcome in, coinfected Egyptian patients. Also highlighted the poor anti-viral therapy outcome among coinfected subjects and proposed several future strategies that may help in resolving the puzzling relationship between human schistosomiasis and HCV infection.

Helminth infections switch immune response to favor secondary viral infection

Helminth infections exert profound suppressive effects on the host's immune response, which enable their worms to evade the defense mechanisms and survive in the blood, lymphatics, intestine, body cavities, or other tissues for years.[65–67] Chronically infected filarial and schistosome patients present clinically with impaired immune responses, as demonstrated by reduced production of IL-5 and IFN-γ, and occasionally IL-4.[68–71] Although some elements of such immune suppression are reversible upon drug-mediated parasite clearance,[70–72] drug treatments do not induce protective immunity against parasite challenge, and individuals can rapidly become re-infected upon re-exposure to the infective stages of these parasites. Therefore, innovative strategies to induce long-term protective immunity and to develop vaccines against helminth infections which can counteract infection-induced immune suppression are still lacking. Recently, serum CCL11 and CCL17 were identified as serological indicators of human multiple helminth infections. They were determined to be primarily driven by *S. mansoni* infection as these biomarkers significantly correlated with fecal bilharzia egg counts and bilharzial induced IL10.[73] Besides modulation of the immune response, helminth infections cause chronic disease, including fatigue, iron deficiency anemia, growth stunting, malnutrition, and poor cognitive development.[74] Both the immune suppressive effects and the severe pathological consequences resulting from chronic helminth infections represent risk factors that increase host predisposition to secondary viral infections.

Schistosomiasis infection induces T_H2 responses that enhance both susceptibility to HCV infection and progression of liver pathology

One possible reason for both enhanced susceptibility to HCV infection and liver pathology is the shift in the immune response and corresponding cytokine release. While mounting a successful immune response against HCV infection would be characterized by a T_H1 immunological profile that triggers a robust antiviral response and a reduction in host fibrosis, schistosomiasis triggers a T_H2 cytokine response, which not only suppresses T_H1 cytokine release (thereby hindering cellular and antiviral immunity) but also promotes subsequent T_H2 responses and fibrogenesis.[15,52,54,75–79] Such suppression of T_H1 cytokines in case of *S. mansoni*/HCV coinfection was reported to be mediated by antigens shed from dead parasite worms or eggs, and therefore did not

Table 1. Candidate schistosome antigens, their capacity to stimulate various host immune responses, and their protective potential against schistosomiasis infection

Antigen	Form	Induced immune response	Reduction of egg count (%)	Reduction of worm count (%)	References
Sm-p80	DNA vaccine	Th1, IgG, IgG2a and IgG2b	84	59	[26]
TSP-2	Recombinant protein	IgG, IgG1 and IgG2a	~65	57	[27,28]
Sm29	Recombinant protein	Th1, IgG, IgG1 and IgG2a	60	51	[29,30]
Sm200 (ECL)	DNA vaccine	IgG, IgG1> IgG2a	ND	38.1	[13]
Sm 25	Peptide vaccine	IgG	Significant	Significant	[32,33]
Glutathione peroxidase	DNA prime vaccinia virus boost	ND	ND	85	[34]
Sm21.7	Recombinant protein	ND	ND	41–70	[35]
Cu/Zn superoxide dismutase	DNA vaccine	ND	ND	44–60	[34]
Filamin	DNA vaccine	Th1/2, IgG, IgG1, 2a and 2b	ND	44–57	[36]
Sm fimbrin + Sm 21.7	Multivalent DNA vaccine	IgG	41.5 (Liver) 55.6 (Intestine)	56	[37]
Sm-p80	DNA vaccine	Th1/Th17, IgG	ND	47	[38]
Sm 23	DNA vaccine	IgG	ND	44	[39,40]
Sm 21.7	DNA vaccine	IgG	62 (liver) 67 (intestine)	41.53	[41]
Fimbrin	Recombinant protein	ND	ND	39.4–41.6	[42]
Sm 22.6	Recombinant protein	Th1/Th2, IgG, IgG1 IgG2a	ND	34.5	[43]
TSP-1	Recombinant protein	IgG, IgG1 and IgG2a	52 (liver) 69 (feces)	34	[26,27]
Stomatin like protein-2	Recombinant protein	Th1 IgG, IgG1 > IgG2a	No significant difference	30–32	[44]
Sm 20.8	DNA vaccine	ND	ND	28.5–30.8	[45]
Sm28GST	DNA vaccine +plasmid containing IL-18	Th1, IgG	29.6% (liver) 27.5% (intestine)	22.6	[46]
Dif 5	DNA vaccine	ND	ND	22	[47]
SmIg	Recombinant protein	Th1/Th2, IgG	ND	No significant difference	[48]
Sm21.6	Recombinant protein	Th1/Th2b IgG, IgG1 > IgG2	ND	No significant difference	[49]

require an active parasitic infection.[34] The shift in immune response from T_H1 to T_H2 can be confirmed by high plasma levels of the profibrotic cytokines IL-4 and IL-13 and anti-inflammatory cytokine IL-10.[75–77,80–84]

Consistent with this, it was reported that impaired interferon gamma (INF- γ) and tumor necrosis factor alpha (TNF- α) production as well as impaired maturation of dendritic cells in a HCV patient who underwent liver transplantation resulted in an inadequate immune response to viral progression and caused relapsing HCV infection.[85] In addition, induction of T- cells from infected humans with human T cell lymphotropic virus type 1 by three recombinant schistosome antigens, namely Sm29, ShTSP2 (tetraspanin), and PIII, caused downregulation of IFN- γ production, which in turn enhanced virus propagation.[86] Similarly, T-cell responses in blood from positive Egyptian patients for both anti-HCV and anti-*Schistosoma* antibodies showed a significant decrease in core-specific T-cell IFN- γ, IL-4, and IL-10 responses compared to T-cells in blood from Egyptian patients positive only for anti-HCV antibodies.[56,76] The authors concluded that the presence of *S. mansoni* eggs in the liver inhibited local intrahepatic T-cell responses against HCV infection and consequently promoted persistent viral infection and accelerated the clinical course of HCV in HCV/*S. mansoni* coinfected humans. This could at that time provide a possible explanation for why coinfected patients have a higher incidence of cirrhosis and HCC than those matched for age, disease duration, and viral genotype with chronic HCV mono-infection.[53]

Immune evasion mechanisms by both HCV and *S. mansoni* proteases enhance relapsing HCV infection and severe liver pathology

Although escape from adaptive immunity is a key to long term persistence of HCV infection, evasion of innate antiviral

responses is crucial in establishing a persistent infection in the first place.[87] As a main player in innate immunity against viruses, the IFN system is key in curtailing pathogens by putting infected and neighboring cells into an antiviral state.[88] HCV has been known for long time to be highly sensitive to treatment with type I IFNs,[89,90] and to date, IFN-α is the major component of HCV therapy. Since HCV is sensitive to IFN and can still manage to establish persistent infection, this suggests that the virus may have evolved mechanisms to circumvent the innate antiviral defense system. In fact, the viral serine protease domain, nonstructural protein (NS) 3/4A, was found to suppress activation of IFN regulatory factor 3 (IRF-3), a central antiviral transcription factor promoting the production of IFN-β and a plethora of IFN-stimulated genes (ISGs), most prominently ISG56,[91] in response to viral infection or treatment with double-stranded ribonucleic acid.[87,92]

Further identification of cellular targets of the HCV protease indicated that it interferes with both Toll-like receptor 3 (TLR3)–mediated signaling through cleavage of the essential adaptor TRIF (Toll/interleukin receptor domain containing adapter-inducing interferon β)[93,94], mitochondrial antiviral signaling protein[95] and TLR-independent activation of IRF-3 by the cytosolic pathways of retinoic-acid inducible gene I (RIG-I) and MDA5 (melanoma differentiation associated protein 5) by inactivating the signaling adaptor CARDIF (also known as interferon-beta promoter stimulator 1 [IPS-1], mitochondrial anti-viral signaling protein [MAVS] and/or virus-induced signaling adaptor [VISA]).[96] This indicated that both pathways may play a role in controlling HCV infection, most likely, in different sets of cells because TLR3 was shown to be essential for IFN production in plasmacytoid dendritic cells, whereas RIG-I triggers IFN secretion upon viral infection in conventional dendritic cells and other tissues.[97] More recently, the preferential abrogation of the RIG-I-mediated pathway following proteolytic cleavage of CARDIF by the HCV NS3/4A protease ectopically expressed in human primary hepatocytes was reported. The TLR3-mediated pathway was affected to a lesser extent and in a protease-independent manner.[98] In support of the impairment of innate anti-HCV responses, IRF3-dependent genes, type IFN-β, type III IL28A/IL29 and chemokine CCL5 were significantly downregulated in association with a global decrease of CARDIF adaptor in liver biopsies and corresponding purified hepatocytes of chronic HCV patients.[99] Altogether, these data suggest that HCV- NS3/4A protease is an attractive anti-HCV drug target, and therefore, I and others molecularly characterized this protease and reported on selective serine protease inhibitors as potential anti-HCV therapeutics.[90,100–103]

A variety of proteases of various classes and clades were also reported to be differentially expressed in various developmental stages of the schistosome parasites.[104–110] These were also demonstrated to be implicated in the evasion of the host immune mechanisms for the sake of establishing chronic bilharzial infection. Cercarial and schistosomular proteases were reported for their capacity to cleave both complement molecules[111–115] and immunoglobulin E[116,117] and in this way prevented host mediated parasite killing by such molecules. Furthermore, helminth cysteine proteases were recently reported to inhibit TRIF-dependent activation of macrophages via degradation of TLR3, which represents an additional immune evasion mechanism by parasite worms.[118]

In murine systems, inhibition of schistosome proteases using either specific inhibitors,[119] newly synthesized small organic molecules[120,121] or by inducing specific immunity against DNA constructs encoding worm proteases[122] resulted in partial protection against *S. mansoni* infection as demonstrated by either reduction in worm burden or egg counts.

Response of HCV/schistosoma coinfected patients to IFN- α therapy

Treatment of HCV with pegylated interferon and ribavirin represents the cornerstone and the standard of care for the management of the prevailing HCV genotype 4a (HCV-4a) in Egypt.[123] HCV-4a has been reported to be one of the most difficult genotypes to treat.[123] One possible reason for this is the great diversity of virus quasi species present in each patient, which provides a reservoir of mutations that enable virus-escape from anti-viral therapy.[124,125] A very recent study conducted on Egyptian patients clearly demonstrated a significant association between positive serology for schistosomal infection and failure to HCV treatment despite anti-schistosomal therapy with praziquantel.[126] This further supports that bilharzial infection complicates HCV disease progression and treatment.

Conclusions

The lack of recent data regarding the actual prevalence of both human schistosomiasis and schistosomiasis/HCV coinfection clearly highlights the urgent need for active surveillance studies for both pathogens in Egypt. I believe that eliminating human schistosomiasis will significantly contribute to the reduction of both HCV prevalence and liver pathology among Egyptians and, as proposed by others, advocate plans for the elimination of human schistosome parasites from Egypt.[127,128] The present review highlights the significant impact of the neglected tropical disease human schistosomiasis on both susceptibility of Egyptian patients to HCV coinfection, severity of the resulting liver pathology, and poor response to antiviral therapy. In addition to the exerted immune evasion mechanisms by the HCV-NS3/4A protease, I discuss for the first time the possible impact of immune evasion mechanisms exerted by proteases of larval, worm, and egg stages of the parasite *Schistosoma* on human susceptibility to HCV infection. In addition, schistosome immune evasion mechanisms are among the suppressive tactics of the parasite that prevents clearance of HCV viremia and leads to both relapsing HCV infection and severe liver pathology.

In fact, developing *in vitro* and *in vivo* models would enable the study of the impact of HCV-4a alone or in combination with live schistosome stages (schistosomula, worms and eggs) on liver pathogenesis and immune modulation. The *in vitro* system requires generation of a replicon system[129] from the most prevailing genotype (HCV-4a) in Egypt and establishment of its replication on hepatoplastoma or immune cells in presence of bilharzial antigens.[60] The ideal *in vivo* system would be a humanized small animal model that can acquire both HCV and *S. mansoni* infections in order to study the impact of coinfection on both the immune system and liver pathology in real-time.[130,131]

Last but not least, both international collaborative and funding initiatives are needed to help resolve the puzzling relationship between human schistosomiasis and HCV infections.

Conflict of interest

None

Author contributions

Writing the review (MMB).

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