Original Article

Studies on the genomic association between schistosomiasis and hepatitis C virus infection

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ABSTRACT

Background: Schistosomiasis is an infection caused by blood flukes of the genus *Schistosoma*. Schistosomes are successful parasites, apparently as a result of prolonged co-evolution with their hosts. Studies done nationwide in Egypt found the highest risk of hepatitis C virus (HCV) co-infection is in those infested with schistosome without history of blood transfusions. However, the association between schistosomiasis and HCV infection is incompletely understood. **Aims:** The overall aim of this study was to assess whether or not a genomic association between schistosomal infestation and HCV infection exists. **Materials and Methods:** Oligonucleotide specific primers of HCV-polymerase chain reaction (PCR) diagnostics were used to screen the genomic DNA and cDNA library pool of *Schistosoma mansoni* as templates based on the end-point PCR approach. **Results:** Screening of schistosome DNA by PCR, lead to the detection of sequences similar to HCV. PCR products were obtained when adult worms genomic DNA were used as templates while no PCR products were amplified from *S. mansoni* λZAPII cDNA library pool. The resulting PCR products were sequenced and compared with the other closely related HCV sequence database at the website < http://hcv.lanl.gov>. **Conclusions:** This work demonstrates the existence of HCV and its replication in the genomic DNA of *S. mansoni*. In addition, it highlights the fact that the parasite can carry the virus genome and therefore, is considered as a nonhuman vector for the transmission of HCV infections.

Key words: Genomic association, hepatitis C virus, polymerase chain reaction, schistosomiasis

INTRODUCTION

Schistosomes are blood flukes mainly belonging to three species of the trematode parasite *Schistosoma*: *S. hematobium*, *S. mansoni* and *S. japonicum*. Less widely distributed members are *S. intercalatum* and *S. mekongi*. *S. hematobium* is the most prevalent of the species and is endemic in Africa and the Middle East. *S. mansoni* infection is endemic in Africa, South America, Caribbean and areas of the Middle East, while *S. japonicum* exists in parts of East Asia.[1] In Egypt, *S. mansoni* occurs intensively in the Nile Delta, while *S. hematobium* is endemic in the entire Nile Valley.[2,3] The World Health Organization (W.H.O.) estimated > 200 million persons in 74 countries are infected of which 85% live in Africa, South of Sahara.[4,5] In the Middle East including Egypt, the most important risk factor of squamous cell carcinoma and bladder tumors is infestation of the bladder by *S. hematobium*.[6,7] Schistosomes become successful parasites as a result of prolonged co-evolution with their hosts. They have evolved several ways to evade the host immune system and other host defenses.[8]

Hepatitis C virus (HCV) belongs to the *Flaviviridae* family and the genus *Hepacivirus*. It is a spherical, enveloped single stranded positive sense RNA virus. The single positive-strand RNA genome contains a large open reading frame flanked by highly conserved untranslated regions at both the 5` and the 3` termini.[9] The virus genome is about 9600 nucleotides. Due to the lack of proofreading by the HCV RNA polymerase, HCV has an exceptionally high mutation rate and therefore a high genetic variability.[10]

W.H.O. estimates that approximately 170–200 million people are infected with HCV worldwide and around 3–4 million are newly infected each year.[11] Egypt has the highest HCV countrywide prevalence ranging from 10% to > 40% among different regions and demographic

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groups.[12-14] HCV infection is an important public health concern because the majority of infections do not resolve, but lead to chronic hepatitis C, one of the most frequent causes of liver diseases.[2,15,16]

Co-infection with schistosomiasis and HCV is verified by HCV-RNA in schistosomiasis positive individuals, and the detection of the HCV-specific antibody (Ab) (anti-HCV) in the serum of patients co-infected with the two.[17,18] The epidemiological situation of schistosomiasis and HCV co-infection among Egyptians nationwide is not clear. Few reports showed that in Egypt schistosomiasis positive individuals are co-infected with HCV as well as demonstrating mortality higher rates than patients with single infection.[18-20] The prevalence of HCV-Ab was reported to be 70% in adults suffering from schistosomiasis and had no prior history of blood transfusion.[2,13] Moreover, the prevalence of HCV within the schistosomiasis infected population is far higher compared to the general population of HCV mono-infected. This is supported by the high HCV loads in the *Schistosoma* infected patients suggesting that HCV could positively be transmitted by *Schistosoma.*

The area of schistosomiasis/HCV co-infection is intriguing and presents many challenges for the future global health. The connection between schistosomiasis and the occurrence of HCV was suggested by Bahgat *et al.*,[21] and also in this work. Several reports attempted to reveal the underlying mechanisms of the schistosomiasis/HCV co-infection. The majority of these have focused on the injections of humans with poorly sterilized syringes and blood transfusions.[2,13]

This study aims to investigate the possibility of the simultaneous schistosomal infestation and HCV infection and the genomic relationship between them, by exploring whether the parasite could be a vector of transmission of the virus to human.

MATERIALS AND METHODS

DNA extraction from adult worms

Adult worms of *S. mansoni* were collected from the *Schistosoma* Biology Supply Center at Theodor Bilharz Research Institute (PO Box 30 Imbaba, Giza, Egypt) and kept in 70% ethanol at 4°C. Genomic DNA of adult worms was extracted by standard procedures. Briefly, the adult worms were kept in lysis buffer (0.1 M EDTA, pH 8.0, 0.1 M Tris-HCl, pH 7.5, 0.2 M NaCl, 1% sodium dodecyl sulfate, 0.2% β-mercaptoethanol, and 100 μg of proteinase K), at 65°C for 2 h. Lysis was followed by using the DNA preparation mini Kit (Jena Bioscience, Egypt) according to the manufacturer's instructions. The DNA white pellets were taken and kept at 4°C until used as templates in polymerase chain reaction (PCR). DNA concentration and purity was measured using a quantitative NanoDrop 1000 spectrophotometer according to the manufacturer's protocol.

Polymerase chain reaction

The DNA templates for PCR assay were the purified genomic DNA sequence extracted from the adult worms (described in this work) and the λZAPII cDNA library pool extracted from *S. mansoni* (Wu *et al.*,[22,23] and were kindly provided by Dr. Philip LoVerde, SUNYAB, USA). The HCV-genome universal specific primer sequence was used. Forward primer (F; 5`-CGCAGAAAGCGTCTAGCCAT-3`) and reverse primer (R; 5`-CTCGCAAGCACCCTATCAGG-3`) were obtained from published sequences,[24] which were designed to specifically anneal to conserved regions within the HCV-5`UTR.[9,21] Other primers sequences described by Ohno *et al.*,[25] designed for highly conservative 5`UTR of the corresponding HCV genome were used. These specific primers were 20 nt sequences str F (5`-AGACCGTGCACCATGAGCAC-3`) and str R (5′-TGTTGCATAATTGATCCCGT-3`). Primers synthesis were performed and ordered from Thermo Hybrid Premier Biosoft International (www.Premier.Biosoft.com).

All PCR amplifications were made in final volumes of 50 μL in a nuclease-free Eppendorf tube with 40 μL of PCR Universal TaqMan Master Mix (Applied Biosystems) containing 0.5 μL each of the two amplification primers at a final concentration of 30 pmoles for each primer and 4 μL of DNA templates (0.078 μg/1 μL). The PCR tubes were placed and carried out in a DNA Thermal Cycler apparatus (Perkin-Elmer). When the temperature in the block reached 95°C and kept at that temperature for 4 min for strand separation. After that, 35 cycles of amplification were performed. The cycle of denaturation at 95°C for 0.5 min, annealing at 58°C for 1 min and nucleotide addition (extension) at 72°C for 1 min. When the last cycle was completed, the reactions were kept for another 8 min at 72°C for a final extension step followed by cooling at 4°C until the tubes were removed from the machine. The amplified PCR products were mixed with a 6X gel DNA loading dye (Fermentas, Cat. No. R0611) and examined by electrophoresis on 2% agarose gels stained with 0.5 μg/ml of ethidium bromide (EB) for visualization under UV light. The DNA bands were documented using a Biorad Gel Doc 1000 system and quantified using Quantity One version 4.2.3 software (Bio-Rad) according to the manufacturer's descriptions. All products obtained were sized using a standard 100-bp DNA Ladder molecular weight (Jena Bioscience GmbH, Cat. No. M214). Positive control PCR reactions were also performed to evaluate the efficiency of the PCR reactions with the same guidelines as mentioned above. Negative controls were included in all PCR-runs to prevent misjudging following the risk of contaminations of the samples.

Direct sequencing of amplified DNA

With the advent of DNA methodologies, like the introduction of PCR and nucleotide sequencing, it has become common practice to sequence the PCR products commercially. We sent the PCR products for sequencing to the Biotechnology Research Center at Suez Canal University, Ismailia 41522, Egypt. Briefly, the amplified PCR products were extracted once with ethanol precipitation after the phenol-chloroform treatment.[26] Direct sequencing of the PCR amplified and purified products was performed with the automatic sequencing assay using the Taq Dye Deoxy Terminator Cycle Sequencing Kit with the ABI 3700 automated DNA sequencer (Applied Biosystems) following the manufacturer's protocol for the sequencing reaction. One strand of the DNA was sequenced. The sequencing results have been further grouped and analyzed. The nucleotide sequences from this study were multiple aligned and edited manually by visual inspection. The sequences from this study were compared alongside with those of HCV reference sequences retrieved from the Los Alamos HCV database (http://hcv.lanl.gov/content/hcv-index).[27,28]

RESULTS

DNA techniques and polymerase chain reaction amplification

Among the total of 21 independent PCR amplification reactions carried out with different primers, only two bands were observed and yielded two sets of the primer sequences including the primer pair str F (5`-CGCAGAAAGCGTCTAGCCAT-3`), str R (5`-CTCGCAAGCACCCTATCAGG-3`), while the other primer set refers to the forward primer (F; 5`-AGACCGTGCACCATGAGCAC-3`) and the reverse primer (R; 5` TGTTGCATAATTGATCCCGT-3`) [Figure 1, lanes 2 and 3].

Analysis of the agarose gel electrophoresis revealed several interesting findings. The amount of amplified PCR products detected by agarose gel [Figure 1] was almost thick and the extent of amplification nearly reached a peak level after 35 cycles of amplification suggesting that the primers set were specific. The optimal concentration of the PCR DNA products for the samples was quantified using a NanoDrop ND 1000 spectrophotometer instrument (Thermo Scientific). All experiments were repeated at least 2 times with the same conditions, and the mean value was used for further analysis. No differences between the amplified DNA concentrations in each of the repeated PCR products were observed as judged by the NanoDrop and the agarose-stained gel results. These results revealed that PCR amplifications induced by the primers set were specific [Figure 1]. Moreover, the amplified PCR DNA products on agarose gel electrophoresis identified nonidentical mobilities and hence represented different sequences.

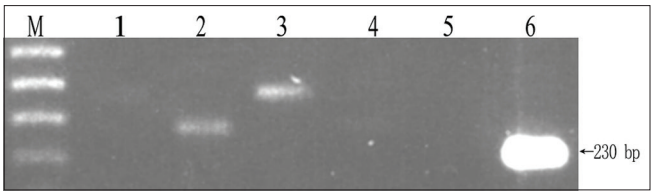

Figure 1: Photograph of agarose gel electrophoresis stained with ethidium bromide showing the polymerase chain reaction (PCR) amplified products from genomic DNA and λZAPII cDNA library pool of *Schistosoma mansoni* as templates using oligonucleotide of the hepatitis C virus-specific primers. M: DNA size marker (Jena Bioscience GmbH, Cat. No. M214). Lane 1: Negative control (no DNA). Lane 2: PCR product of ~240 bp was amplified from adult worms genomic DNA by using primer sequences str F (5`-CGCAGAAAGCGTCTAGCCAT-3`) and str R (5`-CTCGCAAGCACCCTATCAGG-3`). Lane 3: PCR amplified product of ~350 bp from adult worms genomic DNA by using forward primer (F; 5`-AGACCGTGCACCATGAGCAC-3`) and reverse primer (R; 5`-TGTTGCATAATTGATCCCGT-3`). Lanes 4 and 5: Primers failed to amplify any specific DNA sequence when *S. mansoni* λZAPII cDNA library pool is used as a template. Lane 6: Positive amplification control. The arrow points to the ~230 bp band *The data of this figure are mainly derived from the MSc thesis by Tarek M. El-Beltagy (2012)

The high fidelity PCR products yielded only long band sizes of ~ 240 bp and ~ 350 bp on agarose gel electrophoresis including the PCR primers [Figure 1, lanes 2 and 3]. A negative control sample was done without DNA template in order to check for contamination [Figure 1, lane 1]. Other primers failed to amplify any specific DNA sequence when *S. mansoni* genomic DNA or λZAPII cDNA library pool is used as a template. These results indicate that the described PCR system may not be specific for screening of HCV genome or the target sequences were not present when *S. mansoni* genomic DNA and λZAPII cDNA library pool are used as templates. Obviously, we cannot draw any firm conclusions on this point since our study material is small during the study period.

Direct sequencing of polymerase chain reaction amplified products

Direct sequencing of the PCR amplified genomic DNA was performed to confirm the PCR products. Only one PCR amplified product was successfully sequenced using a dilution of the previously described original PCR primers with the sequence str F (F; 5`-CGCAGAAAGCGTCTAGCCAT-3`) and the sequence str R (R; 5'-CTCGCAAGCACCCTATCAGG-3`). The obtained sequencing data were identified and delivered via the HCV database searchable website < http://hcv.lanl.gov>.

DISCUSSION

The area of schistosomiasis and HCV co-infection is intriguing and presents many challenges. This co-infection, will definitely, has a significant impact on global heath. It is of great importance to explore the possible genomic

association between *Schistosoma* and HCV co-infection and the underlying mechanisms.

Co-infection with schistosomiasis and HCV leads to a more-rapid progression of the known pathology of hepatic schistosomiasis expressed as accelerated fibrosis to decompensated liver cirrhosis, end-stage liver disease and hepatocellular carcinoma. All of these conditions are synergized in *Schistosoma*-HCV co-infection resulting in a higher incidence of liver-related morbidity and mortality.[21,29-32]

The PCR technique showed a remarkable sensitivity and specificity when schistosome[33] and HCV[34] examinations were used as the reference test. It is more sensitive and also renders a more-rapid result mainly than isolation and detection in routine diagnosis. In the current study, we investigated the usefulness of the PCR assay to screen the schistosome DNA by using oligonucleotide specific primers of HCV-genome. Furthermore, this PCR based approach was carried out to clarify the possible genomic association between schistosomiasis and HCV infection, through existence of HCV and its replication in the genomic DNA of *Schistosoma* parasite.

The molecular weights of the amplified fragments [Figure 1] produced by the PCR assay from adult worms DNA agreed with previously reported detection of the HCV genome using the same primers using the same primers.[9,24] The absence of any amplification PCR products from negative control sample, also reflects the specificity of the used primers for the detection of HCV-genome when *S. mansoni* DNA is used as a template.

In this work, evidence for viral genetic material and *Schistosoma*-HCV genomic association was confirmed by PCR method. However, these findings need to be verified in a larger prospective study.

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REFERENCES

1. Chitsulo L, Engels D, Montresor A, Savioli L. The global status of schistosomiasis and its control. Acta Trop 2000;77:41-51.
2. Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS, *et al.* The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet 2000;355:887-91.
3. Seif el-Din SH, El-Lakkany NM, Mohamed MA, Hamed MM, Sterner O, Botros SS. Potential effect of the medicinal plants *Calotropis procera*, *Ficus elastica* and *Zingiber officinale* against *Schistosoma mansoni* in mice. Pharm Biol 2014;52:144-50.
4. Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J. Schistosomiasis and water resources development: Systematic review, meta-analysis, and estimates of people at risk. Lancet Infect Dis 2006;6:411-25.
5. Gray DJ, McManus DP, Li Y, Williams GM, Bergquist R, Ross AG. Schistosomiasis elimination: Lessons from the past guide the future. Lancet Infect Dis 2010;10:733-6.
6. El-Bolkainy MN, Mokhtar NM, Ghoneim MA, Hussein MH. The impact of schistosomiasis on the pathology of bladder carcinoma. Cancer 1981;48:2643-8.
7. Tawfik HN. Carcinoma of the urinary bladder associated with schistosomiasis in Egypt: The possible causal relationship. Princess Takamatsu Symp 1987;18:197-209.
8. Morgan BP, Daha M, Meri S, Nicholson-Weller A. Into the third century of complement research. Immunol Today 2000;21:603-5.
9. Choo QL, Richman KH, Han JH, Berger K, Lee C, Dong C, *et al.* Genetic organization and diversity of the hepatitis C virus. Proc Natl Acad Sci U S A 1991;88:2451-5.
10. Dustin LB, Rice CM. Flying under the radar: The immunobiology of hepatitis C. Annu Rev Immunol 2007;25:71-99.
11. Global surveillance and control of hepatitis C. Report of a WHO consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. J Viral Hepat 1999;6:35-47.
12. Abdel-Aziz F, Habib M, Mohamed MK, Abdel-Hamid M, Gamil F, Madkour S, *et al.* Hepatitis C virus (HCV) infection in a community in the Nile Delta: Population description and HCV prevalence. Hepatology 2000;32:111-5.
13. Habib M, Mohamed MK, Abdel-Aziz F, Magder LS, Abdel-Hamid M, Gamil F, *et al.* Hepatitis C virus infection in a community in the Nile Delta: Risk factors for seropositivity. Hepatology 2001;33:248-53.
14. Lehman EM, Wilson ML. Epidemic hepatitis C virus infection in Egypt: Estimates of past incidence and future morbidity and mortality. J Viral Hepat 2009;16:650-8.
15. Al-Sherbiny M, Osman A, Mohamed N, Shata MT, Abdel-Aziz F, Abdel-Hamid M, *et al.* Exposure to hepatitis C virus induces cellular immune responses without detectable viremia or seroconversion. Am J Trop Med Hyg 2005;73:44-9.
16. Ghany MG, Strader DB, Thomas DL, Seeff LB, American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C: An update. Hepatology 2009;49:1335-74.
17. Zaaijer HL, Cuypers HT, Reesink HW, Winkel IN, Gerken G, Lelie PN. Reliability of polymerase chain reaction for detection of hepatitis C virus. Lancet 1993;341:722-4.
18. Kamal SM, Rasenack JW, Bianchi L, Al Tawil A, El Sayed Khalifa K, Peter T, *et al.* Acute hepatitis C without and with schistosomiasis: Correlation with hepatitis C-specific CD4(+) T-cell and cytokine response. Gastroenterology 2001;121:646-56.
19. Darwish MA, Raouf TA, Rushdy P, Constantine NT, Rao MR, Edelman R. Risk factors associated with a high seroprevalence of hepatitis C virus infection in Egyptian blood donors. Am J Trop Med Hyg 1993;49:440-7.
20. El-Sayed NM, Gomatos PJ, Rodier GR, Wierzba TF, Darwish A, Khashaba S, *et al.* Seroprevalence survey of Egyptian tourism workers for hepatitis B virus, hepatitis C virus, human immunodeficiency virus, and *Treponema pallidum* infections: Association of hepatitis C virus infections with specific regions of Egypt. Am J Trop Med Hyg 1996;55:179-84.
21. Bahgat MM, El-Far MA, Mesalam AA, Ismaeil AA, Ibrahim AA, Gewaid HE, *et al. Schistosoma mansoni* soluble egg antigens enhance HCV replication in mammalian cells. J Infect Dev Ctries 2010;4:226-34.

22. Wu W, Niles EG, El-Sayed N, Berriman M, LoVerde PT. *Schistosoma mansoni* (*Platyhelminthes, Trematoda*) nuclear receptors: Sixteen new members and a novel subfamily. Gene 2006;366:303-15.
23. Wu W, Tak EY, LoVerde PT. *Schistosoma mansoni*: SmE78, a nuclear receptor orthologue of *Drosophila* ecdysone-induced protein 78. Exp Parasitol 2008;119:313-8.
24. Blackard JT, Smeaton L, Hiasa Y, Horiike N, Onji M, Jamieson DJ, *et al*. Detection of hepatitis C virus (HCV) in serum and peripheral-blood mononuclear cells from HCV-monoinfected and HIV/HCV-coinfected persons. J Infect Dis 2005;192:258-65.
25. Ohno O, Mizokami M, Wu RR, Saleh MG, Ohba K, Orito E, *et al.* New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J Clin Microbiol 1997;35:201-7.
26. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual. Vol. 18. New York: Cold Spring Harbor Laboratory Press; 1989. p. 42-59.
27. Kuiken C, Yusim K, Boykin L, Richardson R. The Los Alamos hepatitis C sequence database. Bioinformatics 2005;21:379-84.
28. Abdel-Hamid M, El-Daly M, Molnegren V, El-Kafrawy S, Abdel-Latif S, Esmat G, *et al*. Genetic diversity in hepatitis C virus in Egypt and possible association with hepatocellular carcinoma. J Gen Virol 2007;88:1526-31.
29. Kamal S, Madwar M, Bianchi L, Tawil AE, Fawzy R, Peters T, *et al.* Clinical, virological and histopathological features: Long-term follow-up in patients with chronic hepatitis C co-infected with *S. mansoni*. Liver 2000;20:281-9.
30. Elrefaei M, El-Sheikh N, Kamal K, Cao H. HCV-specific CD27- CD28- memory T cells are depleted in hepatitis C virus and *Schistosoma mansoni* co-infection. Immunology 2003;110:513-8.
31. Kamal SM, Graham CS, He Q, Bianchi L, Tawil AA, Rasenack JW, *et al.* Kinetics of intrahepatic hepatitis C virus (HCV)-specific CD4 T cell responses in HCV and *Schistosoma mansoni* coinfection: Relation to progression of liver fibrosis. J Infect Dis 2004;189:1140-50.
32. Farid A, Al-Sherbiny M, Osman A, Mohamed N, Saad A, Shata MT, *et al. Schistosoma* infection inhibits cellular immune responses to core HCV peptides. Parasite Immunol 2005;27:189-96.
33. Pontes LA, Oliveira MC, Katz N, Dias-Neto E, Rabello A. Comparison of a polymerase chain reaction and the Kato-Katz technique for diagnosing infection with *Schistosoma mansoni*. Am J Trop Med Hyg 2003;68:652-6.
34. Baggio-Zappia GL, Hernandes Granato CF. HIV-GB virus C co-infection: An overview. Clin Chem Lab Med 2009;47:12-9.

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