# Coinfection of Schistosoma species with Hepatitis B or Hepatitis C Viruses

Abruzzi, A., Fried, B. and Alikhan, S.B. "Coinfection of *Schistosoma* species with Hepatitis B or Hepatitis C Viruses."

Amy Abruzzi, Edward J. Bloustein School of Planning and Public Policy, Rutgers University, New Brunswick, 08901 NJ, USA (Corresponding author, Amy.Abruzzi@Rutgers.edu)
Bernard Fried, Lafayette College, Easton, PA, 18042, USA
Sukaina B. Alikhan, U.S. Fund for UNICEF, New York 10038, NY, USA

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ABSTRACT

Although a considerable number of studies have been undertaken to date, it is still controversial as to whether or not coinfection with schistosomiasis increases the susceptibility to or progression from HBV or HCV infection. This review is a closer examination of the key studies conducted on human populations on clinical factors that were published in English between 1975 to January 2015. Our review is mainly based on tables containing the salient information, which are arranged first by study population, country of study, and publication date. We provide further explanation, clarification and discussion in the text. As such, it includes both studies that have been conducted on general populations who are largely asymptomatic for clinical disease (Table 1.2.1), as well as those focusing on special populations, which are usually comprised of clinical patients. These special populations have been presented as follow: subjects with chronic liver disease or related conditions such as cirrhosis, Table 1.3.1; subjects with primary liver cancer, Table 1.3.2; subjects with schistosomiasis, Table 1.3.3; subjects with acute or chronic hepatitis resulting from Hepatitis B virus, Table 1.3.4; and, subjects with Hepatitis C virus, Table 1.3.5. We have presented studies that compared two mono-infected groups with one that is coinfected separately in Table 1.4, as these offer us the best basis from which to evaluate if any synergistic effects accompany coinfection.

A number of factors contributed to the results reported in our tables. These included, but are not limited to: subject selection (i.e., asymptomatic cases typically drawn from the general population vs. subjects presenting to a hospital or clinic with clinical disease); study design, which directly impacts our ability to infer causality (i.e., case series, cross-sectional, case control, cohort study); use and choice of control population (i.e., apparently healthy subjects vs. other hospital patients vs. none); sample size, which directly impacts statistical power and can result in a Type II error; geographic area, which may reflect differences in population genetics, public health history, environmental differences or any number of other important factors (i.e., Egypt, Brazil, China); method of testing for schistosomal infections (i.e., stool vs. antibody test); method of testing to determine if advanced schistosomal disease was present (i.e., ultrasound, liver biopsy vs. none); method of serological testing for HBV (i.e., use of HBsAg alone or with other markers or DNA testing); method of serological testing for HCV (i.e., use of anti-HCV alone or with RNA testing); and, year of the study, which reflects among other things, technological improvements between tests as well as possible changes in the frequency of exposure in the populations under study (i.e., use of parenteral anti-schistosomal therapy vs. the oral anti-schistosomal medication).

Despite all these differences, throughout this review we have observed general patterns that seem largely consistent with one another. Studies conducted on general, largely asymptomatic populations tend to support the view that having one of the diseases in question (i.e., schistosomiasis does not necessarily predispose one to becoming coinfected with another (i.e., HBV or HCV). Rather, the probability of becoming coinfected seems most closely associated with modes of transmission for either HBV or HCV in schistosome-endemic areas, such as the past use of parenteral anti-schistosomal therapy or frequent blood transfusion. Once coinfected, however, the clinical course of illness for those with *Schistosoma*-HBV or *Schistosoma*-HCV infections are typically much more severe than for mono-infected subjects. The strongest evidence for this was found in the half-dozen or so prospective cohort studies that systematically monitored disease progression in their subjects. With respect to HBV infection, coinfection with *Schistosoma* prolonged the carriage state and more often resulted in chronic hepatitis with greater cirrhosis as well as higher mortality. Much of the same was also observed with respect to HCV, where coinfection with *Schistosoma* was associated with a reduced ability to spontaneously resolve the viral infection and more often resulted in rapid fibrosis as well as higher mortality. Furthermore, two of these studies which were fully comparative in nature, support the supposition that there is a synergistic association between Schistosoma-HCV for both liver fibrosis and mortality. Immunological studies, all conducted on HCV, also generally seem to support this.

The results of our research argue for greater primary prevention for both HBV and HCV in *Schistosoma*-endemic populations. Although no vaccine currently exists for HCV as it does for HBV, additional steps can still be taken to reduce transmission in high risk populations. Greater use of the HBV vaccine is particularly advisable. Finally, additional observational, longitudinal studies conducted on human populations that are fully comparative in nature could help answer some of the remaining questions on both *Schistosoma*-HBV as well as *Schistosoma*-HCV coinfections. Some of these include the role of active vs. past schistosomal infections, the role of genetic variants, as well as the effect of coinfection on treatment. Future studies should make a particular effort to use a sufficient sample size to ensure adequate statistical power, which was not often properly considered in many of the studies we reviewed for this paper.

KEYWORDS: Schistosoma, schistosomiasis, Hepatitis B Virus, HBV, Hepatitis C Virus, HCV, coinfection, disease progression, interaction, chronic hepatitis, chronic liver disease, hepatocellular carcinoma

Pathogens: *Schistosoma mansoni, Schistosoma japonicum, Schistosoma haematobium*, Hepatitis B Virus, Hepatitis C Virus

Geographical identifiers: Egypt, Brazil, China, Japan, Saudi Arabia, Ethiopia, Kenya, the Philippines, Sudan, United Arab Emirates, Yemen

Abbreviations used Tables 1.1.1- 1.4.1:

Adj, adjusted;

AFP, Alpha-fetoprotein;

ALT, Alanine transaminase;

Anti-HCV, Hepatitis C antibody;

AST, Aspartate aminotransferase;

AVH, Acute viral hepatitis;

CAH, Chronic active hepatitis;

CI, Confidence interval;

CLD, Chronic liver disease;

DHSS, Decompensated hepatosplenic schistosomiasis;

GE, Greater or equal to;

HAV, Hepatitis A virus;

HBsAg, Hepatitis B surface antigen;

HBsAb, Specific antibody to Hepatitis B surface antigen;

HBcAg, Hepatitis B core antigen;

HBcAb, Specific antibody to Hepatitis B core antigen;

HBeAg, Hepatitis B e antigen;

HBeAb, Specific antibody to Hepatitis B surface antigen;

HBV, Hepatitis B virus;

HBV-DNA, Hepatitis B DNA;

HCC, Hepatocellular carcinoma;

HCV, Hepatitis C virus;

HCV-RNA, Hepatitis C RNA;

HDV, Hepatitis D virus;

HDVAb, Antibody to Hepatitis D virus;

HIS, Hepatointestinal schistosomiasis;

HIV, Human immunodeficiency virus;

HGV, Hepatitis G virus;

HSS, Hepatosplenic schistosomiasis;

ICC,Intrahepatic Cholangiocarcinoma;

ISS, Intestinal schistosomiasis;

LC, Liver cancer;

LD, Liver disease;

LE, Less than or equal to;

LSch, Liver schistosomiasis;

LT, Less than;

MHF/MPF, Minimal hepatic periportal fibrosis;

NOS/n.s., not otherwise specified;

OR, Odds ratio;

PAT, Parenteral anti-schistosomal therapy, potassium antimony tartarate

PIIINP, Type III procollagen peptide;

PPF, Periportal fibrosis;

PPT, Periportal thickening;

NA, Not available, unknown, or not specified in original paper;

RR, Relative risk;

Sch, schistosomiasis, schistosome;

SchAb, schistosome antibody;

Sh, *S.haematobium*;

SHF, Schistosomal hepatic fibrosis;

Sj, *S.japonicum*;

SLD, Schistosomal liver disease;

Sm, *S.mansoni*;

SPF, Schistosomal portal fibrosis;

Contents



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## 1.1 INTRODUCTION

This review examines coinfection of selected species of *Schistosoma* with Hepatitis B virus (HBV) or Hepatitis C virus (HCV) in human populations, with an emphasis on the clinical aspects of disease. The schistosomes are water-borne digeneans of global concern that infect humans when they come into contact with a snail-transmitted larval stage (the cercaria) via contaminated water. Infection with schistosomes, particularly the species *S. mansoni* or *S. japonicum*, can result in damage to the liver and more rarely, specific forms of liver cancer. Schistosomiasis has been most often studied in terms of single infections but its role in concomitant infections is of increasing concern, particularly in conjunction with viral infections. HBV and HCV are two such pathogens, infecting nearly 1 in 12 people globally (WHO 2014, WHO 2015; see also Mohd Hanafiah et al. 2013, Ott et al. 2012, ) and of particular interest because of the damage they cause to the liver. HBV is a double stranded DNA virus of the hepadnavirus family, while HCV is a RNA virus with a molecular structure similar to the family of flaviviruses that cause yellow fever or Dengue fever. HVB is often spread through vertical transmission, i.e. mother to child, but may also be spread through horizontal transmission such as through contaminated blood supply. HCV is most commonly spread through contaminated blood supply, and documented high risk groups for HCV include intravenous drug users, health care workers exposed to needle sticks, hemodialysis patients and recipients of blood transfusions; HCV is also often spread through sexual contact and often, patients fall outside of these high risk groups. Chronic infection with either HBV or HCV can result in liver fibrosis, cirrhosis and decompensation. In addition, both of these viruses are associated with primary liver cancer. As with schistosomiasis, the majority of the individuals infected with HBV or HCV live in non-Western countries and may be unaware they are infected; Egypt in especially notable for having a high prevalence of all three infections.

Although a considerable number of studies have been undertaken to date, it is still controversial as to whether or not coinfection with schistosomiasis increases the susceptibility to or progression from HBV or HCV infection (See Gasim 2015, Bahgat 2014, Van-Lume et al. 2013). This review is a closer examination of the key studies to date that are relevant to clinical presentation. As such,

it includes both studies that have been conducted on general populations who are largely asymptomatic for clinical disease, as well as those focusing on special populations, which are usually comprised of clinical patients. The special populations referred to are: subjects with chronic liver disease or related conditions such as cirrhosis; subjects with primary liver cancer; subjects with schistosomiasis; subjects with acute or chronic hepatitis resulting from Hepatitis B virus; and, subjects with Hepatitis C virus. Most all of the studies conducted on general populations, subjects with chronic liver disease, and subjects on liver cancer patients were principally concerned with estimating the frequency of mono and coinfections in these populations. For these tables, i.e. Tables 1.2.1, 1.3.1 and 1.3.2, we have provided a special column reporting data on the prevalence and use a summary column to convey the main findings on coinfection. Many of the studies conducted on subjects with schistosomiasis, subjects with acute or chronic hepatitis from HBV, or subjects with HCV, examined disease severity and progression by contrasting a mono-infected group against those with coinfection. For these tables, i.e. Tables 1.3.3, 1.3.4 and 1.3.5, we have included any exclusion criteria applied to a study population in order to rule out other possible causes of liver infection or hepatitis. In these tables, we report prevalence, when applicable, in the summary column in conjunction with the other results on coinfection. We have chosen to treat studies that compared two mono-infected groups (i.e., Schistosomiasis and either HVB or HCV infection) against a group of coinfected subjects separately from the aforementioned categories. As such, these studies offer us the best basis from which to evaluate if any synergistic effects accompany coinfection.

Many of the studies appearing in Tables 1.2.1, 1.3.1 - 1.3.3 tested for both HBV and HCV in their study populations. Thus, we also present data, whenever available, on coinfection between HVB and HCV, and tri-infection with schistosomiasis in our tables. Finally, a few of the studies included in this review tested for other hepatitis viruses, such as Hepatitis D virus, in their study populations. Hepatitis D virus is a defective hepatropic RNA virus that requires the presence of HBV as a helper virus for its pathogenicity and has been shown to be associated with the most severe forms of acute and chronic hepatitis in many HBsAg seropositive patients (WHO 2015, WHO 2002). People who are immune from HBV are immune from HDV, while carriers of HBV are susceptible to it (WHO 2015). Rather than universally omit this data from our tables, we have noted it when relevant and discuss in conjunction with our findings in the conclusion.

Our review contains numerous tables as in Abruzzi and Fried 2011, in which we examined coinfection of schistosomes with protozoa, bacteria and other helminths, and tabular information is followed by text to clarify and extend the information presented. In order to be included in a table, the study in question needed to meet certain inclusion criteria. First, the study needed to be published in a scientific journal that was indexed by Helminthological Abstracts, MEDLINE or I.S.I's Web of Science from 1975 onwards, or the study needed to appear as a footnote in other studies located through these indexes. Our database search terms were simply "hepatitis and schistosom*", from which we selected studies relevant to either HBV or HCV coinfections. We mainly utilized Google Scholar to double check the results from our database searching and to assist us when following the footnote trail. In addition, the study in question needed to be published in the English language before January 2015, which was our practical limitation. All specific entry numbers are arranged first by the country of the study population and then in

ascending chronological order. No papers were located for this review on species of *Schistosoma* other than *S. mansoni*, *S. haematobium* or *S. japonicum*.

Given the vastness and complexities of the literature, we have only included studies conducted on human populations in this review. Animal studies are sparse on *Schistosoma*-HBV or *Schistosoma*-HCV coinfections, and were excluded. In vitro studies, chiefly using soluble egg antigens, are also beyond the scope of this paper, as are HBV vaccine efficacy studies in schistosome-endemic populations. In order to be included in a table, the study in question need to include sufficient information on the methods used to determine the presence of the coinfection and offer a clear presentation of the results. In each section, we also discuss any relevant data on the mechanisms of coinfection as studied by the specific papers included in our tables. While understanding that the mechanisms of coinfection are obviously important, this was not the primary purpose of our review, as stated earlier. The reader will find additional references to several key papers further discussing the mechanisms of coinfection in our conclusion.

All of the studies included in our tables used observational methods, and many were conducted on clinical patients. In order to better distinguish between the studies vis-a-vis their robustness for inferring causality, we indicated the type of epidemiologic study design used (i.e., case series, cross-sectional, case control, cohort) as well as their primary objective (i.e., prevalence, risk factors, disease progression) in our tables. In some important ways, however, the designs used to study the association between two diseases represent a departure from the traditional exposure-disease paradigm that underlies this epidemiologic classification. As such, our designations are best viewed in this context as points along a continuum of increasing methodological rigor. If the study author(s) did not provide sufficient detail on methods for us to be confident that a higher–level study had been conducted, a lower level designation was applied that adequately described the study in question. When in doubt, we also considered a paper's classification in MEDLINE, which includes descriptors denoting study design when they are clearly demonstrated in the paper. We also considered the classification assigned by Van Lume *et al.* (2013) for the 10 papers they discuss, which we largely agreed with. Papers that presented only a brief account of methods and/or results or were only available by scientific abstract do not appear in our tables, but are occasionally referred to in the text. Case reports or studies relying solely on autopsy were routinely excluded from our review.

The following study design designations were used in our tables and are presented here in order of increasing internal validity or robustness:

Case Series: A case series is a type of descriptive observational study design that closely examines a group of patients with a common set of characteristics, such as a common diagnosis (e.g., patients with schistosomiasis, patients with chronic liver disease, patients who are anti-HCV+), with the aim of further describing their clinical presentation. Typically, the number of cases included in this type of study are small in number and only minor inclusion or exclusion criteria are utilized. In some cases series studies, subjects are selected for inclusion from consecutive patients presenting at a medical facility. Case

series patients may also be followed over time for a change in their disease status, but unlike a cohort study, with no particular disease endpoint mind. Sometimes a comparison population, such as another small group of patients, is used; Occasionally, external control groups are used. In these cases, case series may appear to be like case control studies; However, they lack the same level of rigor with respect to defining case and/or control status as well as control of confounding factors. (For additional discussion, see Kempen 2011).

Cross-sectional: Cross-sectional studies are another type of descriptive, observational study design, which are used to estimate the frequency of disease and its correlation with any exposures of interest in a given study population at a particular point or period in time. In general, cross-sectional studies do not provide evidence of causality, since they measure disease and exposure at the same point in time. However, in clinical studies where exposure measures are valid proxies for past exposures or indicate permanent exposure characteristics, cross-sectional studies and case control studies are largely equivalent (Kramer 1988).

Case Control: Case control studies are a type of analytic, observational design in which a group of subjects who are known to have the outcome of interest (i.e., hepatocellular carcinoma) are first identified as cases. A suitable comparison or control group of subjects without the outcome of interest are then assembled and used for comparison. They are particularly well-suited to studying rare diseases or diseases with long latency periods, and are usually undertaken for the purpose of evaluating the association of specific risk factors with the health outcome of interest. Matching is often used to improve statistical efficiency and to make cases and controls comparable with respect to baseline confounding factors, such as age and sex. Case control studies may be population based and are sometimes carried out in conjunction with other designs, such as a cross-sectional survey; Many of the case control studies included in this review were conducted on hospital or clinic patients. Case Control studies provide some evidence of causality between exposure and disease, provided recall bias was absent or played only a minimal role in ascertaining exposure.

Cohort: A cohort study is an analytic, observational study that selects a group of patients who are initially free of the outcome of interest, records their various exposure statuses, and then follows them over time for the development of that outcome. Prospective cohort studies are one of the best known sub-types of this study design, and are well suited to studying rare exposures and evaluating disease progression, including mortality. The cohort studies cited in this review all began with patients who were diagnosed at an early stage in their infection, then followed over a period of years to systematically monitor the change in their disease status. As such, cohort studies offer us better evidence for inferring a causal relationship between exposure and disease than other observational designs.

There was considerable variety in how schistosomiasis was determined in the study populations in these papers, which we have indicated in our tables and discuss in context. Many studies

routinely checked for ova in stool and/or urine on one or more occasion. When live ova were requisite, we have denoted this in our tables. A substantial number of studies used one or more schistosome antibody test to detect the presence of infection. Notably, this test cannot distinguish past from present infections nor can it always distinguish between *Schistosoma* species. Many studies also used an ultrasound, computerized tomography (CT) scan, and/or liver biopsy to check for fibrosis or other hepatic damage. Most often, studies used a combination of methods, all of which we note in our tables. Many studies also gathered data from prior medical records or by questionnaire, especially for a patient's history of schistosomiasis or past exposure to infested water. We did not include this information routinely in our tables unless this data were used to establish case status in that population. Similarly, since clinical exams including routing blood work and liver function tests were used in the vast majority of these studies, we only noted them in our tables where they were used to define case status or report on them in our comments section when pertinent to major findings. We provide a brief guide to the most commonly used serological markers used in the evaluation of liver disease that are covered in this review in Table 1.1.1

Since most readers of this journal may not be familiar with the serological markers (seromarkers) used to detect the presence of HBV or HCV infections, we provide a brief synopsis in Table 1.1.2. In our tables, we present results for the seromarker(s) used in that specific study. If a combination of markers or other diagnostic measure were used to define disease status in that population, we indicated it. Virtually all of studies in our review tested for HVB by checking for the Hepatitis B surface antigen (HBsAg), which may indicate an acute or chronic infection. Many papers also used one or more additional HBV seromarkers in order to make this distinction, and typically reported data separately for HBsAg seropositivity versus "any HBV marker". Similarly, the vast majority of studies on HCV infection checked for the presence of HCV antibodies (anti-HCV), which may indicate present or past infection. Many studies, especially those conducted in recent years, also conducted tests for HCV-RNA, which indicates the presence of replicating virus. Here, too, there was variation as to if this was used to confirm active cases of HCV infection or was simply gathered as additional information on their study population. It is important to note that the range of studies included in this review were conducted using different serological tests, or different generations of the same test, or conducted in such a way (i.e., repeated tests) that could easily result in varying degrees of sensitivity and specificity. Incorporating that level of detail in our tables and analyzing it accordingly is beyond the scope of this review, which is intended as a broad survey searching for commonalities with suggestions for further study. Finally, when reporting results, we have indicated when non-significant increases were noted. Lack of statistical significance in the context of a single study may be due to lack of statistical power, which is a function of the frequency of the disease in the population under study and number of factors examined. As such, we also indicate the sample size used in each investigation.

Table 1.1.1 Serological Markers Used in the Evaluation of Liver Disease

| Abbreviation | Name and Description |
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|---|---|
| ALT | Alanine transaminase; Also called alanine aminotransferase; One of several liver enzymes routinely examined as indicators of possible liver damage; In healthy individuals, ALT levels are low. |
| AST | Aspartate aminotransferase; Formerly called serum glutamic oxaloacetic transaminase; As with ALT, the presence of higher levels of this enzyme in the blood may be indicative of liver damage; Often tested in conjunction with ALT, and sometimes presented as a ratio of it. |
| AFP | Alpha-fetoprotein; Also written as α-fetoprotein; Widely used as a tumor marker to screen for liver cancer, as well as several other cancers. |

Sources: Rutherford 2014a, Rutherford 2014b, WHO 2014, WHO 2015.

Table 1.1.2 Serological Markers of Hepatitis B Virus or Hepatitis C Virus Infection

| Serological maker | Description |
|---|---|
| Hepatitis B Virus | |
| HBsAg | Hepatitis B surface antigen; Indicates carrier state associated with acute or chronic infection; Often used in the diagnosis of HBV infection and for the screening of blood; This marker is the earliest indicator of acute infection, appearing without HBsAb or HBcAb; Persistence of HBsAg for more than 6 months in conjunction with other markers is indicative of chronic infection. |
| HBsAb | Specific antibody to Hepatitis B surface antigen; Also written as anti-HBs; Appearance after 1-4 months after onset of symptoms is indicative of clinical recovery of and subsequent immunity to HBV; In the absence of HBsAg and presence of HBsAb, indicates previous HBV infection and immunity to hepatitis B; In the absence of both HBsAg and HBcAb, HBsAb indicates vaccine-induced immunity. |
| HBcAg | Hepatitis B core antigen; Marker of infectious viral material; Most accurate index of Hepatitis B viral replication. |
| HBcAb | Specific antibody to Hepatitis B core antigen; Also written as anti-HBc; HBcAb identifies all previously infected persons, including HBV carriers, but does not differentiate carriers from non-carriers; In the absence of HBsAg and HBsAb, this marker indicates a recent HBV infection; Class type (IgM, IgG) used for further distinction. |
| HBeAg | Hepatitis B e antigen; Indicates patient is infectious; Typically appears during weeks 3-6 of infection; Persistence beyond week 10 indicates progression of infection to chronic state; Continuous presence is indicative of chronic active liver disease. |

| | |
|---|---|
| HBeAb | Specific antibody to Hepatitis B e antigen; Also be written as anti-HBeAg; When present in conjunction with HBcAb and in the absence of HBsAg, HBsAb and core HBV mutants, this marker indicates convalescence and low contagiousness. |
| HBV-DNA | Hepatitis B virus DNA; Maybe detectable by hybridization assays or PCR as soon as 1 week after initial infection; HBV DNA polymerase is only performed for research purposes. |
| Hepatitis C Virus | |
| Anti-HCV | Hepatitis C antibody; Usually detected by Enzyme Immune Assay (EIA); Current tests have higher sensitivity and specificity than earlier tests, but additional or confirmatory testing is usually advisable; Individuals will still test positive for anti-HCV, even if they are no longer infected as in the case of spontaneously resolved infections; Alternatively, patients with compromised immune systems may not produce enough antibodies for detection by EIA. |
| HCV-RNA | Hepatitis C virus RNA; Usually detected by polymerase chain reaction (PCR) assay; Presence in serum indicates an active infection; Often used to confirm the diagnosis of hepatitis; Detects disease in patients that may be false negative on anti-HCV, such as immunocompromised patients. |

Sources: Rutherford 2014a, Rutherford 2014b, WHO 2002, WHO 2014, WHO 2015.

## 1.2 STUDIES CONDUCTED ON GENERAL POPULATIONS

This section reviews the studies conducted on general populations where schistosomiasis is endemic for the purposes of measuring the prevalence of coinfection with HBV and/or HCV. The 14 studies selected for inclusion in Table 1.2.1 were conducted in Brazil, China, Egypt, Ethiopia, Kenya, the Philippines, Sudan and Yemen, and were published between 1983 to 2012. Most of the countries in this list are represented by one or two studies; Egypt is best represented with seven. All studies used a cross-sectional design and ranged in size from 242 to 2038 subjects, with about half including less than 700 subjects. With a few exceptions, most were large, population-based surveys conducted in rural village or community settings, typically including males and females from a wide range of ages. A few of these were notable for using random sampling methods to select study subjects (entry numbers 7, 10, 11) or undertaking village or country comparisons (entry numbers 1, 8, 10, 14). In addition, two studies were included in this table that were conducted in Egypt on younger populations: one study was conducted on healthcare workers who were at high risk of workplace exposure to hepatitis viruses through needle-sticks or other forms of contact with contaminated blood (entry number 9); The other on male military inductees presenting for physical examination (entry number 4).

Each study tested for one or more species of *Schistosoma* and either HBV (13 studies) or HCV (8 studies), including seven studies that tested for both HBV and HCV. Most of the studies in this table pertain to *S. mansoni*, with two studies pertaining to *S. japonicum*. Twelve of these studies tested for these presence of *Schistosoma* ova in stool using one or more samples; Five studies also used an ultrasound or other form of sonography to check for advanced disease in their subjects. In addition, five of the Egyptian studies also included a urine test for *S. haematobium*. The two remaining studies used a *Schistosoma* antibody test (entry number 9) and subject recall of past history in conjunction with an ultrasound (entry number 7). Not surprisingly, the frequency of schistosomiasis in *S. mansoni* endemic areas was high, with more than half of the studies finding 49% or more of their populations infected (range: 20% to 71%). In comparison, less than 1% of the population was infected in a non-endemic village in Brazil, which was included as a comparison population (entry number 1). With respect to other species, infection with *S. japonicum* was detected in up to 32% of the study populations in China and the Philippines (entry numbers 2 and 11); Infection with *S. haematobium* was detected in up to 20% of study populations in Egypt based on ova in urine (entry number 4), with most studies detecting it 2% or less of their study populations (entry numbers 3, 5, 6).

All of the studies testing for HBV in this section reported their estimates based on the HBsAg seromarker. A handful of these studies also included additional estimates based on the presence of any HBV marker, which we also reported in our tables. The overall prevalence of HBsAg markers in these studies ranged from less than 1% to 39%, with the higher frequencies reported in China, Egypt and Kenya (entry numbers 2, 8); More often, HBsAg seropositivity was detected less 10% or less of the population (entry numbers 1, 3-7, 9, 10, 12). Among studies testing for a wider range of HBV seromarkers, evidence of past or present infection was found in 24% to 54% of the study population (entry numbers 2, 3, 5, 10, 12). Infection with HCV, as indicated by anti-HCV seropositivity, was rarely found in Ethiopia (1%-3%, entry number 10) or the Sudan (2%, entry number 13), and more often found in Egypt where it was detected in 10% to 40% of study populations (entry numbers 5, 6, 7, 8, 9). The studies that tested both HBV and HCV usually found a portion of their populations coinfected. This ranged from less than 1% to 5% depending in part on if the HBsAg or any HBV marker was used in conjunction with anti-HCV seropositivity (entry numbers 5-7, 9).

*Schistosoma*-HBV coinfections were detected in 1% to 9% of study populations based on HBsAg seropositivity, and 12% - 20% based on any HBV marker (entry numbers 2-6, 11, 12). *Schistosoma*-HCV coinfections, based on anti-HCV seropositivity, was detected in 2% to 11% of the village based study populations in Egypt (entry numbers 5-7). A greater proportion (24%) of *Schistosoma*-anti-HCV+ coinfection was found among Egyptian Health Care workers, but it should be noted that this study tested for schistosomiasis using the antibody test whereas the other studies used stool samples, sometimes with ultrasound. Coinfection with *S haematobium* and HBV was generally not reported. The study with the highest S haematobium prevalence (20%) found 2% of their study population coinfected based on HBsAg seropositivity (entry number 4). Similarly, coinfection with *S. haematobium* and anti-HCV+ was not reported presumably due to no or few cases (entry number 6). None of the studies in our table that tested for both HBV and HCV reported the proportion of tri-infected individuals. In addition to these studies, El-Esnawy and Al Herrawy

(2000) surveyed 233 male wastewater workers in Egypt, ages 20 to 60 years of age. Coinfection with HBV or HCV and *Schistosoma* as indicated by antibody status was common, and was detected in 16% and 40% of the workers, respectively. In addition, 9% of these men appear to have been triple infected with HBV, HCV and schistosome antibody positive.

Overall, studies did not find an association between HBV and *S. mansoni* or *S. japonicum* across the entirety of their study populations, typically when comparing the proportions of HBsAg seropositivity in those with schistosomiasis against those without coinfection. (entry numbers 1-4,10-12,14). An increase was noted for HBsAg seropositivity among children with *S. haematobium* (entry number 14), however, this appears to be mainly due to one particular village in a multi-village study; A non-significant increase in the proportion of HBsAg among *S. haematobium* positive recruits was also noted among the young male military recruits (entry number 4). Typically, studies also found no statistically significant difference when any HBV marker was used (entry numbers 2, 5, 6, 11, 12); Only one study noted a non-significant increase of HBV coinfection among individuals infected with *S. mansoni* (40% vs. 33%, entry number 3).

In addition to estimating prevalence, a number of the studies in this table examined if coinfection correlated with the severity of disease (entry numbers 1, 2, 4-8, 10-12). With respect to HBV, several studies that analyzed patients with advanced schistosomiasis separately from the general study population reported an association with coinfection. A higher proportion of HBsAg seropositivity was noted among subjects with advanced *S. japonicum* (43%) infection or among those reinfected with *S. japonicum* (23%), when compared to those with a cured (17%), recent (12%) or no infection (16%) (entry number 2). A similar pattern was observed when any HBV marker was used. Another study found an increase among subjects with *S. mansoni* related schistosomal periportal fibrosis/thickening based on either HBsAg (OR 3.5, 95% CI 1.9-6.7) or any HBV marker (OR 2.1, 95% CI 1.4-3.3), with a 40% higher risk found among subjects with the heaviest S mansoni egg counts. (entry number 10). In addition, two other studies reported non-significant increases. In Entry number 5, a tendency was noted for subjects with schistosomal fibrosis to be coinfected with HBV and/or HCV, while in entry number 11, the frequency of HBsAg seropositivity increased with the severity of *S. japonicum* parasitism. As with HBV, studies did not tend to find an association between anti-HCV seropositivity and schistosomiasis across the entire study populations (entry numbers 5, 6, 8, 10), and to a lesser extent, did for those with advanced disease in Egypt. One study conducted found a small increase in risk of anti-HCV+ among those with schistosomal periportal fibrosis (entry number 7), while another noted a non-significant increase among those with the same condition in another (entry number 5). No association was found, however, in another study examining subjects with more generally defined hepatocellular damage (entry number 8). Finally, Tavares-Neto et al. (1998; 2005) did not find any associations with either HBV or HCV and schistosomiasis in the investigations they conducted in Brazil, which included analyses by type and severity of *S. mansoni* infection.

A studies few gathered additional data with the aim of better elucidating the timing and/or mode of transmission of the relevant infections (entry numbers 4, 6, 7, 9, 10, 12, 13). Some of the studies in this section suggest that infection with schistosomiasis occurs at a younger age in endemic areas, prior to HBV or HCV infection, which more often occurs later in life (entry numbers 6, 7, 10).

As one study noted, adults aged 40 and over were infected four times more often than children with HBV (entry number 6). In addition, another study observed that HCV infection appeared to reach its peak prevalence at a younger age (60% by age 30) than HBV (75% by age 40) in their study population (entry number 7). Exceptions to this would be populations where HBV is more often acquired through birth, in which case coinfection with schistosomes would occur after (see discussion in entry number 12). There has been a commercially available vaccine for HBV since the 1980s, which has likely reduced the prevalence of this virus in some populations, and therefore coinfection (see entry 9, which indicated that almost 2/3 of the health care workers under study had been immunized; see also Ott et al. 2012). Currently, there is no effective vaccine to prevent HCV infection, which along with reduced control of schistosomiasis, may have increased the frequency of coinfection in others (see Guerra et al. 2012, Mohd Hanafiah et al. 2013, Sanghvi et al. 2013). Of note, a number of the studies that were conducted in Egypt reported an increased frequency of HBV or HCV seromarkers among subjects who received parenteral anti-schistosomal therapy (PAT; also described as potassium antimony tartarate), which was an older, injection based treatment for schistosomiasis in use prior to the development of oral-based treatment (i.e., praziquantel ). In this table, PAT was associated with increased risk for HBsAg seropositivity (entry number 4) as well as for anti-HCV seropositivity (entry number 6). Less directly, another study found that HCV status was associated with a past history of schistosomiasis, which was in turn associated with PAT (entry number 7). The association was not found in entry number 13, conducted in the Sudan. The association with PAT was raised in several other studies in this review, and will be addressed in our conclusion.

## 1.3 STUDIES CONDUCTED ON SPECIAL POPULATIONS

This section concerns studies conducted on special populations, typically patients with clinical liver disease. They vary with respect to if any of the pathological agents responsible for the disease were unknown or known at the time the study was undertaken. Most often, studies used a cross-sectional design to estimate the frequency of the infections among study subjects or a case-control design to estimate the risk associated with the infections for a particular health outcome. Cases series designs were also fairly common, with a handful selectively following patients over time. Less often, prospective cohort studies were used to carefully monitor and assess progression of disease in one or more groups of patients (see Tables 1.3.3, 1.3.4, and 1.3,5).

The studies presented in Tables 1.3.1. and Tables 1.3.2 were conducted on subjects with chronic liver disease or related conditions, or on subjects with primary liver cancer. The studies in both of these tables selected patients who were unknown with respect to schistosomiasis as well as HBV or HCV status. Since these studies were undertaken in part to estimate the prevalence of these infections in their patients, we present this data in a separate column in our tables.

The studies presented in Tables 1.3.3 and 1.3.5, were conducted on patients previously diagnosed with schistosomiasis or with HCV, respectively. As such, chronic patients figure prominently in these studies, and as a general rule a wider range of diagnostic methods were utilized. Some of the papers discussed here were seeking non-invasive biomarkers that could be used instead of

liver biopsy for prognosis. A number of papers look at immunological aspects of coinfection with schistosomiasis, particularly when combined with HCV. The studies in Table 1.3.4 are a mixture of the types discussed above. In all of these studies, however, the hepatitis that is tested and reported upon is HBV, often with additional data on disease severity. As mentioned earlier, a few of the studies in this review also tested for and reported on HDV. Rather than omit it, we include it in our tables and discuss it where it is relevant.

### 1.3.1. SUBJECTS WITH CHRONIC LIVER DISEASE AND RELATED CONDITIONS

The eight studies in Table 1.3.1 were undertaken in Egypt, where chronic liver disease (CLD) is usually attributed infection with *Schistosoma* mansoni. Typical feature of hepatic schistosomiasis, which may also be called schistosomal liver disease, include the development of hepatic granuloma and periportal fibrosis, with bleeding from gastroesophageal varices. For reasons that are not always clear, many patients with CLD have preserved liver functions, while others have a more progressive course and die from hepatic failure and or/complications including hepatocellular carcinoma. These studies, published between 1995 and 2002, were undertaken to investigate if coinfection with HBV and/or HCV infection may explain some of these differences.

Of the eight studies in this table, one was a case series (entry number 2); the other seven were either cross-sectional (entry numbers 1, 3, 4, 6) or case control studies (entry numbers 5, 7, 8). With respect to the cross-sectional studies, two used external controls drawn from blood donors in some analyses (entry numbers 4 and 6); One cross-sectional study also gathered additional data on subjects including history of PAT or blood transfusion. All of the case control studies used healthy controls matched for age and sex (entry numbers 5, 7 and 8); One case-control study included a second control group comprised of chronic disease patients (entry number 5), while another study matched cases and controls by neighborhood (entry number 8).

Overall, the studies in this table ranged in size from 46 to 1023 subjects, with the majority of studies conducted on 250 or fewer subjects. In addition to being Egyptian, study subjects tended to be male (60-80%), with mean ages that ranged from approximately 30 to 48 years. Most subjects were drawn from patients who presented at a hospital or clinic with symptoms that included recurrent jaundice, chronic hepatitis, ascites, and a history of gastrointestinal bleeding. Sometimes, but not always, persistently elevated serum ALT levels were noted (i.e, entry numbers 3, 6). Two of the studies restricted their patients to those with either minimal hepatic periportal fibrosis (entry number 2) or liver cirrhosis (entry number 7).

Most studies checked for ova from *S. mansoni* in stool samples and/or rectal snip, with a few using a schistosome antibody test as an alternative; In addition, one study also specifically checked for *S. haematobium* infection (entry number 6). The proportions infected with schistosomiasis in these populations depended on whether it was detected by based only on stool and/or rectal snip (8% to 32%, entry numbers 1,3, and 8), or relied in whole or in part on the presence of antibodies (66% to 84%, entry numbers 2-5, 7). Entry number 3 was notable for reporting results based on both methods. Two studies focused on active schistosomal infections (entry number 1, 3). Among

controls, the proportion infected with schistosomiasis was not always reported; When it was, it varied widely, ranging from 15% to 64% in two studies both testing for schistosomal antibodies. (entry numbers 4, 5). Most subjects were then examined by ultrasound and/or liver biopsy, from which it was determined if they had one or more of the following: periportal fibrosis, splenomegaly, hepatic decompensation or cirrhosis, or hepatocellular carcinoma.

All eight studies conducted serum tests for the HCV antibodies and/or HCV-RNA, six of which also tested for the presence of HBV usually using the HBsAg marker. HCV was particularly common among patients with more generally defined CLD, which ranged from 54% to 75% based on anti-HCV seropositivity (entry numbers 1,3,4,6, 8). A comparable proportion was found for one of the studies using HCV-RNA as their serological indicator (74%, entry number 5), while a somewhat lower proportion was reported by another study based on their methods (43%, entry number 8). HCV infection was detected least often in the two studies conducted on minimal hepatic periportal or liver cirrhosis patients, which reported 26% (entry number 2) and 24% (entry number 7) of their patients were anti-HCV seropositive, respectively. The frequency of HCV infection was not always reported for control populations depending on the nature of the study. When it was, it varied widely depending on control population, with 0%, 14% and 47% of controls testing anti-HCV seropositive (entry numbers 7, 4, 8, respectively), and 6% to 43% based on HCV-RNA seropositivity (entry numbers 5, 8). HBV infection was as found far less often, with 6% to 16% of study populations testing HBsAg seropositive (entry numbers 1-3,5, 8). HBsAg seropositivity was even more rare among controls, infecting approximately 2% or fewer subjects (entry numbers 5, 8); Finally, several studies reported the proportions of their patients who were coinfected with HVB-HCV, which ranged from 3% to 7% (entry numbers 1-3, 5). Only one study also noted that HBV-HCV coinfection occurred among their controls (4%, entry number 8).

Six of the studies in this table reported the frequency of coinfection with HCV in their study population, which appeared to vary depending on several factors including patient population and methods of testing for schistosomiasis. Among CLD patients, studies identifying stool based, active schistosomiasis infections detected coinfection with HCV in 6% to 10% of their populations (entry numbers 8, 3, respectively), whereas studies that utilized a schistosome antibody test to identify past or present infections found 41% to 63% of their populations coinfected (entry numbers 3, 4, 5). The proportions did not appear to vary based on study design or whether anti-HCV and/or HCV-RNA testing was used. Among controls in these studies, the proportion coinfected with HCV was 6% for stool based, active infection (entry number 8, case control) and 10% to 22% when based on schistosomal antibody test (entry numbers 4, 5, cross sectional and case control, respectively). With respect to related conditions, *Schistosoma*-HCV coinfection was detected in 22% of liver cirrhosis patients (entry number 7, case control) and 10% of patients with minimal hepatic periportal fibrosis (entry number 2, case series). Both of these studies relied on a schistosome antibody test, with one study using it as an alternative to stool and or/rectal snip based-samples (entry number 2).

Overall, all of the studies that analyzed disease severity among patient groups, found it associated with *Schistosoma*-HCV coinfection. Coinfected CLD patients displayed more severe liver disease than non-coinfected patients, with greater portal hypertension and /or cirrhosis. (entry numbers

1, 3, 5, 6). Several cross-sectional studies connected coinfection to the presence of live *Schistosoma* ova in either the stool or rectum. One study found that coinfected patients were more likely to have active *S. mansoni* infection (82%) than patients without eggs (68%) or, with dead eggs in their rectum (63%) (entry number 1). In another, coinfected patients with active *S. mansoni* infection had greater cirrhosis and hepatic malignancies (entry number 3). Similarly, coinfected patients with active HCV, as detected by the presence of HCV RNA, found it associated was a greater severity of liver disease. (entry number 3, 6; See Figure 1) Among cirrhosis patients studied using a case control design, coinfection was associated with enhanced nitric oxide levels, which increased proportionately with the severity of disease. (entry number 7).

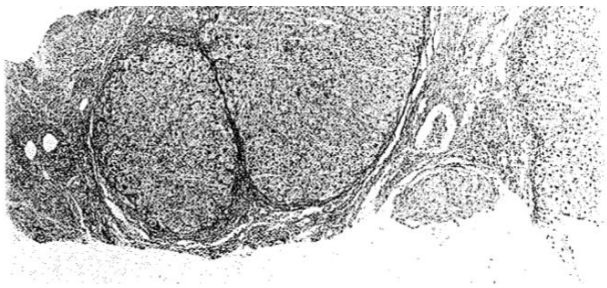

Figure 1. From Angelico et al. 1997: "Liver biopsy from an anti-HCV positive patient excreting schistosomal eggs in stools. The microphotograph shows parenchymal nodules surrounded by fibrous septa. Inflammation is observed within portal tracts, septa and at the stroma-parenchyma interface (cirrhosis with features of chronic aggressive hepatitis)"



Fewer studies reported data on *Schistosoma*-HBV coinfections, which were considerably less common than with HCV. Among CLD patients, coinfection was detected in 2% of patients with active *S. mansoni* infections and 10% when the antibody test was used (entry numbers 3 and 5, respectively); Coinfection was not found among apparently healthy controls in the one case control study which reported data on it (entry number 5). Only one of the cross sectional studies analyzed patients by disease severity, finding coinfected patients had greater portal fibrosis and cirrhosis (entry number 3). Notably, these coinfected patients all had active *Schistosoma* disease. Contrary to this, past history of schistosomiasis rather than active disease was associated with coinfection with HBV; No other data were provided on these patients. (entry number 8, case control) Finally, neither coinfection with schistosomiasis and either HBV or HCV was associated with MPF in the case series reported in entry number 2.

### 1.3.2 SUBJECTS WITH PRIMARY LIVER CANCER

This section examines studies conducted on subjects with primary liver cancer, with most studies focusing on hepatocellular carcinoma (HCC). HCC occurs more often in males, typically aged 50 years or older, and has a higher incidence in Africa and Asia with half of all global cases occurring in China (Jemal et al. 2011). HCC is strongly associated with scarring of the liver (cirrhosis), which may be caused by a number of factors including alcohol abuse and autoimmune disorders of the liver. In developing/non-Western countries in particular, HCC is most often associated with HBV or HCV infections. (Jemal et al. 2011). One of the studies included in this section examines coinfection among patients with Intrahepatic Cholangiocarcinoma (ICC), which is a rare subtype of primary liver cancer that is also known as bile duct cancer. Bile duct cancer of the intrahepatic variety frequently emerges in the setting of chronic liver disease where it requires differential diagnosis with respect to HCC. (Bragazzi et al. 2012) Difficult to diagnose, it frequently presents at a late stage when no effective therapeutic intervention is possible. (Bragazzi et al. 2012) The presence of gallstones in biliary ducts of the liver (i.e., Hepatolithiasis) and HCV infection are established risk factors for ICC, while at present hepatic schistosomiasis, liver cirrhosis and HBV infection are regarded as probable causes (Bragazzi et al. 2012).

The six studies in Table 1.3.2, dated 1984-2010, were conducted in China, Egypt, Japan and Saudi Arabia. Most of the studies in this table were conducted on middle aged, male hepatocellular carcinoma patients (entry numbers 2-6) and ranged in size from 33 to 102 patients. Four of the studies presented in this table used a case control design, with controls comprised of disease free subjects of comparable age and sex (entry numbers 1, 3-5); Two studies used matching to better balance these possible confounders (entry numbers 1 and 6). Three of the four case control studies used multivariate methods to estimate risk and checked for the presence of statistical interaction between key factors (entry numbers 1, 4 and 5). The remaining two studies used a case series design to evaluate the frequency of coinfection, one of which included a minimally described control group (entry numbers 2 and 6).

In all of the studies, liver cancer was histologically confirmed, either through a biopsy done at the time of the study or previously as determined by a review of the patient's medical records. Two of the six studies pertained to infections with *S. japonicum*; The other four studies all pertain to *S. mansoni* and/or *S. haematobium* infections. The majority of these studies relied on a schistosome antibody test to determine infection; Only one of the case control studies checked stool and urine for evidence of current *Schistosoma* infections (entry number 3). Among HCC patients in *S. mansoni* and *S. haematobium* areas, the prevalence of *Schistosoma* infection was 59% based on ova in stool/urine (entry number 3) and 21% to 36% in studies testing for schistosome antibodies (entry numbers 4,6). The prevalence of schistosomiasis among the controls in the case control studies ranged from 12% based on stool/urine (entry 3) to 14% based on an antibody test in the one study that tested for it (entry number 4). In *S. japonicum* areas, the prevalence was 57% among HCC patients based on a schistosome antibody test, which was comparable to the frequency observed among their controls (58%, entry number 5). Evidence of liver schistosomiasis due to *S. japonicum* was slightly higher, however, among ICC patients than their controls (5% vs. 1%, entry number 1).

All six of the studies in this table tested for the presence of the HBsAg marker. Three of these studies (entry numbers 1, 2, 4) also tested for anti-HCV seropositivity, with one also testing for the presence of HCV-RNA (entry number 2). The frequency of HBsAg among HCC patients in these studies ranged from 11% to 58% (entry numbers 2-6), compared with approximately 3% in any reported control population (entry numbers 4, 5). Among ICC patients, HBsAg was found in 49% of patients and 7% of controls (entry number 1). The frequency of HCV infection was higher than observed for HBV among HCC patients, which were found to be 76% (entry number 4) and 94% (entry number 2) anti-HCV seropositive. Notably, both of these study populations were in Egypt, where coinfection between HVB and HCV was also found in 16% of the study population (entry number 2). Among controls, 43% were found to be anti-HCV seropositive in the only case control study report such data (entry number 4). Compared with HCC patients, the frequency of HCV among ICC patients was considerably lower, occurring in less than 1% among ICC cases and absent in controls (entry number 1).

Few studies reported the frequency of coinfection in their study populations. Of the two case control studies that did, one study found 9% of HCC patients and 2% of ICC patients were coinfected with HBV and schistosomiasis (entry numbers 3, 1, respectively). All studies conducted analyses of their data for coinfection, however, and found an association between HBV and schistosomiasis (entry numbers 1, 3, 5, 6) or between HCV and schistosomiasis (entry numbers 2, 4). Among HCC patients, the frequency of HBsAg was higher among *Schistosoma* patients with ova in stool and/or urine than among those without the parasitic infection (15% vs 5%, entry number 3); Similarly, the frequency of HBsAg seropositivity among HCC patients was higher among those tested positive for schistosome antibodies than among those who tested negative in a case series study (66% vs. 53%, entry number 6). As mentioned earlier, several case control studies used multivariate methods to estimate the risk associated with specific factors. One study found that schistosomiasis (OR 5.2, 95% CI 2.9-9.3) in conjunction with HBV (OR 12.5, 95% CI 6.1-25.6) elevated the risk of HCC over that observed for HVB alone (entry number 3). The absence of a reported interaction suggests this effect may be additive. Elsewhere, a multiplicative interaction was noted for the risk of HCC, this time associated with coinfection with S. japonicum and HBsAg infection in conjunction with the daily consumption of 1 cup or more of Japanese Alcohol (RR 10.0, 95% CI not reported, entry 5). Autopsy studies of HCC patients have also suggested an association between S. japonicum and HBV (Nakashima 1975; Kojior et al. 1986). Both HBsAg seropositivity (RR 9.7, 95% CI 6.3-14.8) and liver schistosomiasis (RR 11.1, 95% CI 3.4, 36.3) were also found to be independent risk factors for ICC, again without interaction, suggesting an additive rather than a multiplicative effect (entry number 1).

Finally, two studies in this section (entry numbers 2, 4) evaluated the effects of *Schistosoma*-HCV coinfection among HBsAg negative subjects. The first of these was a case series, and found that *Schistosoma* antibodies occurred more often in anti-HCV positive patients than in controls who were also anti-HCV seropositive, which could not be attributed to other likely factors such as alcohol abuse, hormone use or greater toxin exposure (92% vs. 61%, entry 2). The other study followed a case control design (entry number 4) and estimated an interaction between anti-HCV+ and Schistosome antibody seropositivity (OR 10.2, 95% CI 1.3, 79.8) that was greater than the sum of anti-HCV+ (OR 6.5, 95% CI 1.6,26.6) and Schistosome antibody seropositivity (OR 0.2, 95% CI

0.1-6.2) alone, using a multivariate model. In addition, a higher proportion was also reported by El Tonsy et al. (2014), who found that 61% of the anti-HCV HCC patients they examined were coinfected with schistosomiasis based on an antibody test. In this study, he also found that coinfected patients had a younger mean age and more often had tumors that were multifocal and larger in size than in subjects with HCV alone. This, in conjunction with the other results reported above, suggest a more aggressive course of disease for coinfected subjects.

### 1.3.3 SUBJECTS WITH SCHISTOSOMIASIS

The 30 studies in this table were all conducted on patients with schistosomiasis, many with an advanced form of the disease, and ranged in publication date from 1976 to 2013. Egypt and Brazil are best represented, with 12 and 6 studies each, respectively. The remaining studies were conducted in China, Japan, Kuwait, Saudi Arabia and the Sudan, and are each represented by two or three studies. Accordingly, most these studies in this table pertain to the *S. mansoni* species. Ten of the studies also tested for *S. haematobium* ova in the urine. Nine of these were conducted in Egypt, in populations where *S. mansoni* infections were more common; The remaining study was conducted on Egyptians in Kuwait and focused exclusively on urinary schistosomiasis (entry number 25). In addition, five of the studies in this table pertained to *S. japonicum* infection (entry numbers 7 - 9, 22 and 23). The vast majority of these studies used multiple tests to determine the presence and extent of schistosomal infection. These methods routinely included checking for the presence of ova in stool and/or in the rectum through rectal snip, the use of the schistosomal antibody test, and the use of an ultrasound and/or a liver biopsy to check for the presence of granuloma and evaluate the extent of damage to the liver. Only a few studies relied on stool and/or urine checks (entry number 8 and 13) or the schistosomal antibody test (entry number 18) as the sole or main method. Several studies in this table reported that all of their study subjects had viable ova in their stools (entry numbers 1, 12 and 15). Just as often, studies indicated that only a portion of their subjects had viable ova, even after multiple samples were checked (entry numbers 9, 21, 23, 27). Usually these subjects were at an advanced stage of schistosomiasis, when the inflammatory reaction and scarring of the intestinal wall is such that it can prevent deposited eggs from moving into the intestinal lumen and exiting through the stool (Li *et al.* 2011).

The studies in this table ranged in size from 9 to over 900 study subjects, about two thirds of which were conducted on patient groups of around 100 or less; Most compared disease severity between coinfected and mono-infected schistosomal subjects and were careful to exclude subjects with other possible causes of chronic liver disease, including alcohol abuse and other hepatitis viruses other than those of interest. Eleven of the studies used a case control design (entry numbers 1, 13, 16-21, 25-27), two of which matched controls by sex and age. There were also eleven studies using a cross-sectional design (entry numbers 2-4, 6,8,9,11,14,15,22,28) as well as six studies best described as case series (entry numbers 5, 7, 23, 24, 29 and 30). Many of the cross sectional (entry numbers 2-4, 22 and 28) and case series (entry numbers 5, 29 and 30) studies used a control group in one or more analysis, which were typically comprised of blood donors, medical staff or occasionally a selection of other patients. Only two of the studies in this section followed a prospective cohort design (entry numbers 10 and 12), which was used to evaluate the progression of disease. Several of the case control studies included patients at

various stages of schistosomal disease, and so were able to make additional comparisons pertaining to coinfection (i.e., entry numbers 16, 19 and 20). A few of the studies, chiefly case control, compared mono and coinfected subjects for immunological or genetic differences (entry numbers 20-22 and 25).

More than half of the studies in this table tested for the presence of HBV (21 studies) in their schistosomiasis patients; Fourteen of the studies tested for HCV, including five that tested for both HBV and HCV. In the studies concerned with HBV infection, the HBsAg seromarker was used most often to determine infection with data on any additional HBV markers reported separately. In the studies concerned with HCV, infection was always determined by the presence of the anti-HCV seromarker, sometimes with additional testing for HCV-RNA. Overall, many of the using a non-schistosomal control group found a higher proportion of both HBsAg in their schistosomal patients. This seemed to vary less by study design than it did by country, severity of schistosomiasis in the patient population and composition of the control population. In two cross-sectional studies conducted in Brazil, HBsAg seropositivity was found in 8% to 10% of schistosomiasis patients spanning various stages of the disease, compared with 0% to 2% of other patients who were used as controls (entry numbers 2 and 4). The proportion of HBsAg seropositivity in schistosomal patients versus non-schistosomal controls was usually higher in most other countries, respectively: Japan (14% vs. less than 1%, cross-sectional, entry 22); Saudi Arabia (26% vs. 4%, case-control, entry number 26); Sudan (30% vs. 15%, case series, entry number 29); Egypt (37% vs. 3%, case control, entry number 16). Occasionally, differences in the frequency of this marker were not statistically significant, particularly in some of the smaller case series studies (i.e., entry number 30, 22% schistosomal patients vs. 4% hospital controls). In at least one other small study, this time a case control, the proportion of patients and controls infected with HBsAg was identical (10% vs. 10%, entry number 25).

The studies that tested for HCV were even more consistent in their findings, all reporting greater anti-HCV seropositivity among schistosomiasis patients than in their control populations and spanning a range of study designs (entry number 3, 5, 18, 22, 25, 28). This generally ranged from 13% to 35% in most studies. It was appreciably higher among schistosomal patients with elevated ALT levels (53%, cross sectional, entry number 21) and among active urinary schistosomiasis cases (70%, case control, entry number 25). Studies conducted in countries other than Egypt usually found less than 2% of controls were anti-HCV seropositive; In these studies, which included case control, cross-sectional and case series designs, the controls were comprised of volunteer blood donors or they were other non-schistosomal patients (entry numbers 3, 5, 22, 25, 28). A much higher proportion of HCV infection was found among controls in a case control Egyptian study, specifically replicative virus, which reported 30% of hemodialysis patients and 20% of the general population attending the hospital for routine checkups were infected (entry number 18). Many countries were represented by at least one study that tested for the presence of both viruses in their study populations. Studies conducted in Brazil, Kuwait and Japan all found greater anti-HCV seropositivity than HBsAg seropositivity in their study subjects (entry numbers 5, 6, 22, 25). The main exception to this was China, where a cross-sectional study conducted on advanced S. japonicum cases who underwent a splenectomy found 44% HBsAg seropositive compared with 6% anti-HCV seropositive (entry number 9). Coinfection with both HBV and HCV was generally not

reported in these studies; Again, the only exception was entry number 9, which reported one coinfected case. Unfortunately, none of the studies conducted in Egypt that were located for this table (entry numbers 10 to 21) tested for both HBV and HCV in the same study population.

The most interesting findings of the studies reviewed in this section came from comparing mono and coinfected schistosomal groups. We begin by reviewing the findings for coinfection with HBV, which typically found that HBsAg seropositive schistosomal patients had more severe disease, as indicated by greater fibrosis, greater cirrhosis, chronic hepatitis or liver cancer, than mono-infected schistosomal subjects across every type of study design (entry numbers 1, 2, 5, 7, 9-12, 14). The association of HBV coinfection with greater liver fibrosis and inflammation in schistosomal patients was particularly well-illustrated in entry number 9, which was conducted on patients with advanced S. japonicum infection (See Figure 2). Studies were particularly consistent in finding an association between HBV and decompensated liver disease (entry numbers 1, 2, and 5). In one of the cross-sectional studies, 83% of subjects with decompensated hepatosplenic schistosomiasis were HBsAg seropositive; A greater proportion of these patients also had replicating virus than the other groups, further supporting the supposition that HBV infection plays an important role in disease progression in schistosomal patients (entry number 2). Notably, two of the studies in this table were prospective cohorts with follow up periods that lasted for up to 2 or 4 years, and both found greater progression of disease among the patients who were coinfected (entry numbers 10 and 12). Mortality was also higher among the coinfected in these two prospective studies (11% of the coinfected in entry number 12, 64% of the coinfected in entry number 10), compared with no deaths among mono-infected schistosomiasis subjects. Similarly, a case series found a high proportion of patients (43%) who died of advanced *S. japonicum* infection in China were HBsAg seropositive; In this study, coinfection was also often found in patients with the poorest liver functions (64%) or in patients with hepatocellular carcinoma (62%) (entry number 7). More generally, coinfection with HBV was associated with greater derangement of liver functions in a number of cross-sectional and case control studies, in particular higher serum ALT, AST and bilirubin levels (entry numbers 15, 22, 26). Greater amounts of vomiting and nausea were also sometimes noted among coinfected patients (entry number 15).

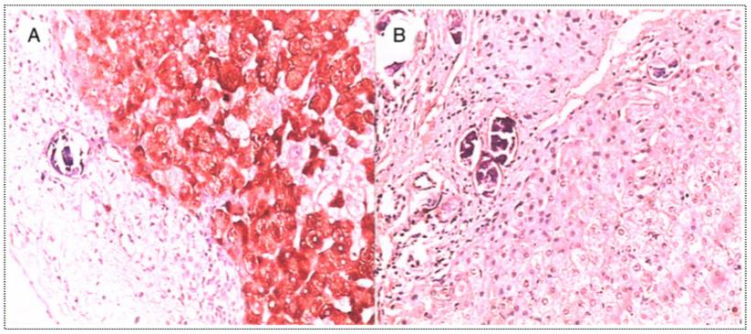

Figure 2. From Li et al. 2011: "HBV infection in liver section shown by immunohistochemical staining of HBsAg. A., Patient with both advanced schistosomiasis and HBV infection. The brown granules present in the cytoplasm denote an active HBV infection (x400 magnification). B., Patient with advanced schistosomiasis only. No brown granules are evident in the cytoplasm (x400 magnification)."

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858
859 In contrast with the results reported above, two case control and two cross-sectional studies did
860 not find an association between disease severity and coinfection with HBV (entry number 4, 8, 13,
861 16). Of interest, two of these studies examined only subjects' stool or urine to determine the level
862 of schistosomiasis infection, and both reported that none of their study subjects had sought
863 medical care for their disease (entry numbers 8 and 13). This seems to imply that none of the
864 subjects in these studies were experiencing advanced, clinical disease. These studies, one cross
865 sectional and the other case control, were also both village-based whereas most of the other
866 studies in this table were conducted on hospital patients, making them an interesting point of
867 comparison with the studies conducted on general populations that we presented earlier in Table
868 1.2.1 Entry numbers 4 and 16 each compared clinical forms of schistosomiasis, usually intestinal
869 schistosomiasis with hepatosplenic schistosomiasis, and neither found a significant differences in
870 the frequency of HBsAg seropositivity between their schistosomal groups. Entry 4, which used a
871 cross-sectional design with a comparison group, reported that patients with the hepatosplenic
872 form had a higher predominance of HBV markers and presented with more severe clinical disease,
873 including greater cirrhosis and worse prognosis than the other groups. Entry 16, which used a
874 comparative case control design, failed to provide additional data on study subjects that would
875 have assisted us in making a better evaluation of its findings.
876
877 Most studies that examined HCV found that coinfected schistosomal patients had more severe
878 liver disease than mono-infected schistosomal subjects, with greater cirrhosis, decompensated
879 disease or hepatocellular carcinoma (entry number 3, 5, 22, 23, 24, 27). The proportions
880 coinfected were often dramatically high and indicative of active infection with HCV. For example
881 in a matched case control study conducted on chronic schistosomiasis patients in Brazil, 81% of
882 subjects with decompensated disease were anti-HCV+ compared with 12% of those with less
883 severe infection; This study also tested for HCV-RNA, and found that overall 62% of chronic
884 schistosomiasis subjects also had active HCV infection (entry number 3). A Brazilian case series
885 reported an even higher proportion of their decompensated hepatosplenic schistosomiasis
886 patients had active, RNA-confirmed, HCV infection (82%, entry number 5). Similarly, 83% of male
887 schistosomiasis patients with cirrhosis in Kuwait tested anti-HCV seropositive on repeated tests;
888 In this case series study coinfected patients also had the greatest severity of disease as indicated
889 by Child-Pugh score (entry number 24). In a case control study conducted in Saudi Arabia,
890 hepatosplenic schistosomiasis patients who were anti-HCV positive had greater cirrhosis (58%)
891 and hepatocellular carcinoma (10%) than those who were anti-HCV negative (entry number 27).

The authors of this study also noted that coinfected subjects also had a lower mean age than mono-infected subjects, suggesting that HCV may result in faster progression to severe disease. This was also supported by a case series that followed 9 chronic schistosomiasis patients in Japan for up to 12 years and found that hepatocellular carcinoma only developed in coinfected cases (entry number 23). Of related interest, Iida *et al.* 1999 documented that a slightly greater proportion of patients developed hematoma who were triple infected with *Schistosoma*-HCV-HBV compared than did *Schistosoma*-HCV patients without the concomitant HBV infection (33% vs. 26%); These proportions were based on a comparison of two small groups, with 9 and 31 patients respectively, and were not statistically significant.

Consistent with these findings, several studies also reported that coinfected subjects had abnormally elevated ALT and AST liver enzyme blood levels, which are usually indicative of greater liver damage (entry numbers 19, 22, 25, 27). This includes the only study in our review conducted exclusively on patients with active urinary schistosomiasis (entry number 25). In this case control study, 22% of patients coinfected with anti-HCV+ had elevated ALT levels, while levels among all mono-infected urinary schistosomiasis patients were normal. The proportion of patients displaying liver enzyme derangement was greater when the subject was coinfected with *S. mansoni* or *S. japonicum* and HCV. In a case control study conducted in Saudi Arabia, for example, 83% of patients with hepatosplenic schistosomiasis had elevated ALT levels compared with 23% of mono-infected subjects, with values that were two to five times the upper limit of what is generally considered normal (entry number 27). An exception to this was the case control study conducted in Egypt, which reported normal liver enzymes in coinfected patients with HCV-RNA (entry number 18). In addition, two of the more recent case control studies in this table examined immune responses or looked for genetic variants that might be associated with the disease severity among the coinfected (entry numbers 20 and 21, respectively). As discussed in more detail in section 1.3.5, patients with *Schistosoma*-HCV coinfection displayed greater Th0-Th2 and lesser Th1 responses compared with mono-schistosomal patients, which appears to favor the chronic form of both diseases and may play a role in their persistence and severity (entry number 20). The second of these studies, also conducted in Egypt, checked for the presence of a genetic mutation that may increase HCV susceptibility in schistosomal patients, but failed to find an association; As in most other Egyptian studies, the anti-HCV seropositive patients were mainly genotype 4a (entry number 21). In comparison, genotype 1a and 3a are usually found in Brazil, and are frequently found coinfected subjects (Alverado-Mora et al. 2012).

### 1.3.4 SUBJECTS WITH ACUTE OR CHRONIC HEPATITIS FROM HEPATITIS B VIRUS

The four studies in Table 1.3.4 all pertain to coinfection between schistosomiasis and Hepatitis B, and were conducted on patients with acute or chronic hepatitis. Acute hepatitis is generally defined in these studies and elsewhere as an inflammation of the liver that lasts less than six months, while chronic hepatitis lasts for longer than this period. Most studies began by testing for the presence of both HBV and schistosomiasis in their hepatitis patient population and compared frequencies between groups. As noted earlier in this review, there are causes other than HBV or HCV that can produce inflammation of the liver. For example, these include other infections such as mononucleosis, chicken pox, as well as drug or alcohol abuse, toxins, fatty liver

disease, trauma, and autoimmune hepatitis (WHO 2014). It is not always clear why some individuals with viral hepatitis recover during the acute stage, while others progress to the chronic form of the disease.

The four studies in this table ranged in date from 1977 to 2014, and includes one of the oldest studies in our review, as well as one of the most recent. The studies ranged in size from 54 to 406 subjects, which tended to be male and represented a range of ages. The three older studies in this section were all conducted in Egypt; The most recent study is from Brazil (entry number 1). Two of the studies were prospective cohorts undertaken, at least in part, to see if coinfection with schistosomiasis plays a role in the progression and severity of Hepatitis B (entry numbers 2, 3). In these studies, subjects were selected with acute viral hepatitis and followed over time to see if they developed chronic hepatitis and evaluated for other complications. The other two studies used a cross-sectional or a case control design, the latter in conjunction with a case series analysis with a modest amount of follow up, to evaluate if the frequency of coinfection in their subjects was associated with disease severity (entry numbers 1, 4).

All of the studies used HBsAg as the main seromarker of interest. Three of the papers also used additional markers or methods that reflect some of the advancements made in detecting HBV infection during this time period (entry numbers 1, 3, 4). All of the subjects who were diagnosed with schistosomiasis in these studies had histological confirmation of *S. mansoni* ova based on stool or rectal snip, with most studies also obtaining a liver biopsy. The three studies based in Egypt also checked for the presence of *S. haematobium*, which was generally absent or rare in these study populations.

Despite a span of more than 20 years in publication dates and a number of other differences, most studies found about one third of their study populations were coinfected with *S. mansoni* and HBV, with frequencies ranging from 31% to 37% (entry numbers 1, 3, 4). Entry 2, one of the oldest studies in this review, reported that 22% of their cohort was coinfected. The four studies were also largely in agreement with respect to findings on disease progression and severity. In one of the cohort studies following acute hepatitis patients over time (entry number 2), coinfected subjects had a greater duration of antigenemia (mean 95 days +/- 143 days) than those testing HBsAg seropositive alone (mean 36 days +/- 61 days). Interestingly, this study noted that a greater proportion of schistosomiasis was not always found among those who were HBsAg seropositive (entry number 2). This was interpreted by the authors as indicating that subjects already suffering from schistosomiasis are not by nature more susceptible to HBV. Once infected with HBV, however, patients with an underlying schistosomal infection appear to have a tendency to remain chronically infected and experience greater disease progression than mono-infected schistosomal subjects. Of particular concern, treatment for the underlying schistosomiasis in this study, did not shorten the carriage rate of HBV observed in these patients.

In the other prospective cohort (entry number 3), the HBsAg carrier rate was nearly 4 fold higher among the coinfected when compared to mono-infected HBV acute hepatitis patients after 1 year of follow up. In addition, coinfected patients were found to have greater splenomegaly, more persistent and greater liver function abnormalities with accompanying histological changes, and

higher mortality than mono-HBV subjects. This finding is echoed by entry number 1, which also found that coinfected patients had more severe liver fibrosis than mono- infected HBV patients (44% vs. 26%); This cross-sectional study also reported that patients with replicative HBV and schistosomal portal fibrosis had more advanced fibrosis and severe inflammation than any other cases. Finally, three of the four studies in this table tested for the presence of Hepatitis D in their populations and two reported its frequency in coinfected subjects (entry numbers 3, 4). The proportions that were triple infected were of note in both of these studies, approximately 9% of all patients with acute viral hepatitis in entry number 3 and 13% of all chronic active hepatitis patients in entry number 4. Entry number 4, which used a case control design for its main analysis, also noted that patients who were triple infected showed the greatest alterations in liver profile, displayed the most advanced liver disease, and had the highest mortality.

### 1.3.5 SUBJECTS WITH HEPATITIS C VIRUS

The 16 studies selected for inclusion in Table 1.3.5 were all conducted on subjects with HCV infection, and all but one was conducted on Egyptians. The studies ranged in publication date from 1998 to 2014, and included a greater share of more recent publications than some of our other tables. More than half of these studies used case control (entry numbers 2, 3, 5, 6, 8, 10 and 12) or cohort (entry number 1 and 7) designs, which typically compared carefully selected groups of HCV mono-infected subjects with subjects coinfected with schistosomiasis versus other controls. The remaining studies were all cross-sectional (entry numbers 4, 9, 13-16), with the exception of one case series (entry 11). Most of the studies in this table were undertaken for purposes of evaluating immunological or other physiological differences associated with coinfection. Severity of disease and other complications were often examined in the studies using case control and cross-sectional designs, while disease progression was evaluated by the two prospective cohorts (i.e., entry numbers 1, 7). A few studies, two of which used a case control design, were undertaken for the purpose of evaluating a non-invasive alternative to liver biopsy that could be used to monitor disease severity (i.e., entry numbers 5 and 12).

Compared with others in this review, the studies in this table also tended to be conducted on a small number of patients, with carefully selected study populations and exclusion criteria. The largest among them was a cross-sectional study conducted on 231 subjects (entry number 13); The vast majority of the other studies involved less than 100 patients. Most were particularly careful to exclude patients with HBV, HDV or other liver conditions, and several noted if their data were gathered prior to subjects receiving standard treatment. In terms of the case controls, controls were typically comprised of similarly aged individuals; Four of these studies used matching to balance age and sex confounders, and occasionally other factors (entry numbers 2, 3, 6 and 8). As elsewhere, study subjects in this table tended to be male, and mean ages between 41-48 years were common. The two prospective cohorts were notable for having study populations with particularly younger mean ages (28 years, entry number 1; 29 years, entry number 7), as was appropriate since substantial follow-up time was involved.

All of the studies in this section began with subjects with confirmed HCV infections. Typically, studies used more than one test to check for anti-HCV seropositivity and confirmed active status

with HCV-RNA. Most studies were conducted on chronic HCV patients typically infected with the virus for at least 6 months (entry numbers 2,3,8,11-13), or patients with active disease that had been present for an unknown or unspecified amount of time (entry numbers, 4,6,9,10,14,16). The two cohort studies both followed patients diagnosed with acute hepatitis subjects over time (entry numbers 1 and 7). None of the other studies in this table were longitudinal, though the one case series (entry number 11) included 6 months of follow up on HCV patients, and two of the cross-sectional studies made use of data collected during other time periods in their in their write-ups (entry number 14 and 16).

With respect to *Schistosoma* species, the fourteen studies that were conducted in Egypt as well as the one from the United Arab Emirates were principally concerned with *S. mansoni* coinfections; The one study conducted in Japan utilized a population based cross-sectional design and pertained to *S. japonicum* (entry number 16). All of the studies used a schistosome antibody test on their subjects, always in conjunction with other diagnostics such as stool, rectal snip, ultrasound and/or liver biopsy, depending on purpose of the investigation. Studies also varied as to whether schistosomiasis was a current or past infection, in part reflecting the difference in design between a cohort and case control study (i.e., entry number 7 vs. entry number 12, respectively). Only a few of the cross-sectional studies estimated the frequency of coinfection in their patients, as most studies began by selecting study groups for comparison. Among those reporting prevalence, estimates varied, with *Schistosoma*-HCV coinfection detected in 25% of chronic HCV patients (entry number 13, Egypt) and 62% and 57% of active HCV disease patients (entry numbers 9, 16, from Egypt and Japan, respectively).

We begin by discussing the two prospective cohort studies, both by Kamal *et al*., that were conducted to evaluate disease progression (entry numbers 1 and 7). Both studies selected acute HCV patients who were then followed over time for a period ranging from about 6 to 8 years, and used a paired liver biopsy at the beginning and end in conjunction with other measures. At the start of each study, coinfected patients had higher viral titers than the mono-HCV groups, but were otherwise comparable with respect to age, sex, extent of fibrosis and other important indicators. In entry number 1, coinfected patients had active schistosomiasis whereas in entry number 7, active disease was not present. Nevertheless, their results with respect to disease progression were remarkably similar. At follow-up, entry number 1 found that 33% of mono-infected HCV patients had resolved their infection compared with 0% of the coinfected; When severity of disease was compared, the coinfected had dramatically higher rates of fibrosis progression compared to mono-infected HCV subjects (0.53 vs. 0.1 units per year, respectively). Similarly, in entry number 7, coinfected subjects also had more rapid fibrosis than mono-infected HCV subjects (0.61 vs. 0.1 units per year, respectively). The coinfected also had developed evidence of portal hypertension with splenomegaly and esophageal varices, independent of liver fibrosis. These results appear to be in keeping with many of the cross-sectional and case control studies in this table that observed greater pathology among the coinfected when compared to their mono-infected HCV subjects (entry numbers 4, 5, 8, 12, 13, 16). The highest risk observed was noted in a cross-sectional study conducted on HCVRNA+ patients (entry number 4), which estimated a 660% increase in the risk of hepatic fibrosis was associated with an active *Schistosoma*- active HCV

concomitant infection (Odds Ratio 7.6, 95% CI 1.9-35.5). Higher levels of fibrosis among coinfected subjects were also described in Hano *et al.* 2011.

As mentioned earlier, many of the studies in this section were undertaken to examine immunological differences and many compared the cytokine profiles of study subjects. Most studies were in agreement, observing that *Schistosoma*-HCV coinfected subjects had dominant Th2 responses while mono-infected HCV subjects had dominant Th1 responses. Two case control studies found that coinfected subjects had lower IFN-gamma and higher IL-10 levels than mono-infected HCV subjects (entry numbers 3, 6). One of these studies also noted higher IL-4 levels among coinfected subjects, but noted that these were not correlated with IL-10 levels or with viral load (entry number 6). In another case control study, IL-10 polymorphisms were not associated with grade of inflammation, stage of fibrosis or responsiveness to combination therapy for HCV infection (entry number 10). Interestingly, in a cross-sectional study coinfected subjects were also found to have a lower prevalence of cryoglobulinemia than mono-infected anti-HCV seropositive subjects, which was attributed to the tipped Th2 immune response associated with schistosomiasis coinfection (entry number 9).

In keeping with the immune responses described above, coinfected subjects were also found to have fewer early CD4+ T cells than mono-infected patients, which was associated with greater disease progression in one of the cohorts we described earlier (entry number 1); A case-control study found fewer late differentiated HCV-specific CD8+ T cells than mono-infected subjects, which was associated with greater pathogenesis (entry number 2). At least two studies reported higher mean TNF-alpha levels in coinfected subjects, indicative of an increased inflammatory response (entry numbers 7 and 12); One of these was a prospective cohort, which found it to be associated with the increased rate of fibrosis observed in coinfected patients (entry number 7). A case-control study reported that coinfected subjects had higher levels of serum actin A in conjunction with a reduction in IGF-1, which was associated with more severe liver disease and a higher risk of hepatocellular carcinoma (entry number 5). Lower IGF-1 as well as IGFBP-3 were found among coinfected subjects in another case-control study (entry number 8), where they served as early predictors of hepatic dysfunction and were associated with other indicators of more severe liver disease. Finally, an association with hepatocellular carcinoma was also noted in entry number 16, a population based cross-sectional study, which found that coinfected subjects developed HCC nearly twice as often as mono-infected HCV subjects (45% vs. 23%).

Two studies appear to be in direct disagreement with the results discussed above (entry numbers 14, 15), and both were cross-sectional in design. One study was an update to Abdelwahab et al. 2012 (Table 1.2.1, entry number 9), which retested asymptomatic anti-HCV seropositive health care workers with known schistosomal antibody status (entry number 14). Overall, mono and coinfected subjects were found to have comparable levels of viral clearance, HCV-RNA titers, and indicators of liver inflammation, though several non-statistical differences such as higher periportal fibrosis among the coinfected were noted. It was not clear, however, how much time had elapsed between the anti-HCV tests reported on in this study, only that the patients had not yet received treatment prior to being retested. The other study, conducted on Egyptian patients in the United Arab Emirates, reported no significant differences between coinfected and mono-

infected patients with respect to the severity of their hepatic pathologies (entry number 15). Neither of these studies used any external comparison populations, nor were noteworthy for utilizing any additional methods that would have strengthened the level of inference that could be drawn from them.

## 1.4 STUDIES COMPARING SUBJECTS WITH SCHISTOSOMIASIS AND SUBJECTS WITH HEPATITIS C VIRUS

The eleven studies selected for inclusion in this table were published between 2000-2011. All but two of the studies were conducted in Egypt. The other two were conducted in Brazil. All of the studies were small, conducted on 100 or fewer subjects, and all focused on schistosomiasis and HCV infections. Ten out of the eleven studies used a case control or cohort design to compare two mono-infected groups with one that is coinfected, as well as a non-diseased control group for comparison. The remaining study was a case series that also compared a small series of patients, but lacked sufficient detail on inclusion or exclusion criteria or other methods to justify a case-control designation. We did not identify any studies for inclusion in this table on schistosomiasis and HBV that followed this type of comparative design and met our other inclusion criteria.

As in Tables 1.3.4 and 1.3.5, studies were often undertaken to compare disease severity, immunological and/or genetic differences, and most studies were careful to eliminate subjects with other viral infections and liver diseases from their study population. All of the studies in this section used stool-based exams to check for ova from *S. mansoni*, often in conjunction with ultrasound to evaluate advanced disease. Three of the eight case control studies used matching to control differences by age and sex (entry numbers 4, 6, and 8); Matching was also used in one of the cohort studies, to make patient groups more comparable with respect to age, sex and duration of infection (entry number 5). A few studies conducted in Egypt also checked for ova in the urine from *S. haematobium*, which was generally absent (entry numbers 3, 10, 11). Two case control studies reported that all of their patients had ova in stool samples (entry numbers 8 and 9); This is in contrast with two other case control studies that reported ova in less than half of their schistosomiasis patients (entry numbers 10 and 11). With respect to testing for HCV, eight of the studies used HCV-RNA to confirm disease status. Based on this testing, three case control studies specifically studied chronic HCV patients (entry numbers 4, 6 and 7). The other, a prospective cohort, specifically followed acute anti-HCV patients for disease progression (entry number 5).

The two prospective cohorts in this section each followed patients for more than 6 years (entry number 3 and 5). The first of these studies (entry number 3), found that over the observation period, coinfected subjects had greater progression of disease, resulting in higher liver related mortality (48%) compared with mono-HCV (12%) or mono-schistosomal (3%) subjects. Hepatocellular carcinoma only developed in coinfected subjects (11%), and not in either mono-infected group. Of interest, most coinfected subjects were HCV Genotype 4 (92%) compared with 62% of mono-HCV subjects. Coinfected subjects also had higher HCV titers and long duration of HCV than mono-HCV subjects in this study. Unfortunately, not all subjects were at the same stage

of disease at the start of this study, which makes additional comparisons difficult. The other prospective cohort in this section (entry number 5), also found greater disease progression in coinfected subjects. In particular, liver fibrosis was greatly accelerated in coinfected subjects with 0.58 units per year compared with 0.1 units per year in mono-infected HCV patients. Few mono-schistosomal patients had any progression of fibrosis, suggesting that the effects are multiplicative in coinfected subjects, rather than additive. Compared with mono-HCV subjects, coinfected subjects also had higher degrees of interface hepatitis, periportal necrosis and a lower magnitude and breadth of intrahepatic HCV-specific CD4+ T cells responses. The authors of this study suggested that the enhancement of progression of liver fibrosis is associated with the failure to develop HCV-specific Th1 responses, particularly during the early phase of chronic infection.

As noted in a number of other studies in this review, distinctive cytokine profiles, particularly tipped towards the Th2 response, were identified for coinfection subjects. Most of the results in this section were studies using case control designs. In entry number 4, coinfected subjects had IL4 and IL10 levels that were comparable to or higher than those observed in mono-schistosomal subjects, and IFN-gamma and IL-18 levels that were considerably lower than mono-HCV subjects. This study suggested that infection with *Schistosoma* preceded HCV infection in coinfected subjects, which inhibited the ability of coinfected subjects to mount HCV-specific Th1 responses. This same cytokine pattern, with IL 4 and IL10 levels meeting or exceeding those observed in mono-schistosomal subjects and IFN-gamma levels below those observed for mono-HCV subjects, was reported in both entry numbers 6 and 8. In addition, entry number 6 also reported that coinfected patients had higher titers of HCV-RNA with reduced CD4+ T cell response, which were also noted in the prospective cohorts discussed above (entry numbers 3 and 5, respectively). Higher IL4 levels were also found for coinfected subjects in entry number 10, a case control study which found them correlated with greater portal vein diameter, more pronounced fibrosis, and greater portal hypertension.

Taken together, these studies suggest that the dominance of the Th2 response observed in coinfected patients may result in increased viral replication, and is likely to be related to the greater fibrosis observed in these patients than in either HCV or schistosomiasis alone. Regardless of study design, all of these studies were in agreement that coinfected subjects do not respond to HCV infection the way that mono-HCV subjects do, the latter typically demonstrating a strong Th1 response. In addition, at least one study (entry number 8) indicated that coinfected subjects displayed lower levels of Th1 cytokines than observed among healthy controls as well as mono-schistosomiasis subjects, suggesting that coinfected suffer from additional immunologic suppression or impairment. In addition, most of the case control studies, but not all, reported that the coinfected subjects had higher mean ALT and mean AST levels than either mono-infected groups, which was often correlated with degree of fibrosis (entry number 4, 6, 7, 10, 11). These findings appear to be consistent with those reported by Wahib et al. 1998, Kamal et al. 2001 and El Shazly et al. 2001, which were not included in our table. Finally, one case control study found that coinfected subjects had a higher frequency of a heterozygote mutant of the Lymphotoxin-alpha genotype; More generally, Lymphotoxin-alpha is a member of the TNF superfamily which may be associated with increased susceptibility to HCV (entry number 11).

In contrast with the findings reported above as well as those described in section 1.3.5, one study in this section reported that coinfected patients had higher TNF-alpha levels than either mono-schistosomal or mono anti-HCV patients (entry number 1). Notably, this study used case series design and provided little data on patients, making it difficult to access their comparability. The results of this study still suggested, however, that immunoregulation of coinfection differs from each disease in isolation. One of the case control studies found no difference in the degree of fibrosis between coinfected subjects and either mono anti-HCV seropositive or mono-hepatosplenic schistosomiasis patients based on evaluation by liver biopsy or ultrasound, respectively (entry number 2); It should be noted that 91% of the schistosomal subjects in this study presented with severe fibrosis. Coinfected subjects did, however, display higher fibrosis markers such as alkaline phosphatase, bilirubin and gamma globulin than other mono-infected groups. It should also be noted that this particular study did not use matching between cases and controls, and lacked additional detail on possible confounders between the comparison populations.

## 1.5 CONCLUDING REMARKS

In this review, we have been concerned with identifying the clinical effects of coinfection between *Schistosoma* and HBV or HCV. A number of factors contributed to the results reported in our tables. These included, but are not limited to: Subject selection (i.e., asymptomatic cases typically drawn from the general population vs. subjects presenting to a hospital or clinic with clinical disease); Study design, which directly impacts our ability to infer causality (i.e., cross sectional vs. prospective cohort study); Use and choice of control population (i.e., apparently healthy subjects vs. other hospital patients vs. none); Sample size, which directly impacts statistical power and can result in a Type II error; Geographic area, which may reflect differences in population genetics, public health history, environmental differences or any number of other important factors (i.e., Egypt, Brazil, China); Method of testing for schistosomal infections (i.e., stool vs. antibody test); Method of testing to determine if advanced schistosomal disease was present (i.e., ultrasound, liver biopsy vs. none); Method of serological testing for HBV (i.e., use of HBsAg alone or with other markers or DNA testing); Method of serological testing for HCV (i.e., use of anti-HCV alone or with RNA testing); And, year of the study, which reflects among other things, technological improvements between tests as well as possible changes in the frequency of exposure in the populations under study (i.e., use of parenteral anti-schistosomal therapy vs. the oral anti-schistosomal medication).

Despite all these differences, throughout our tables we have observed general patterns that seem largely consistent with one another. As has been noted elsewhere (i.e., Gasim 2015, Bahgat 2014, Van-Lume et al. 2013), studies conducted on general, largely asymptomatic populations tend to support the view that having one of the diseases in question (i.e., schistosomiasis does not necessarily predispose one to becoming coinfected with another (i.e., HBV or HCV). Rather, the probability of becoming coinfected seems most closely associated with mode of transmission for either HBV or HCV in schistosome-endemic areas. In Table 1.2.1, several cross-sectional studies reported an increased risk for both HCV as well as HBV from the use of parenteral anti-schistosomal therapy (see entry number 4, Hyams et al. 1987; entry number 6, El-Sayed et al.

1997; entry number 7, Darwish et al. 2001). These findings were echoed in a number of the other studies presented in various tables (see Gad et al. 2001, Strickland et al. 2002) and have been much discussed elsewhere (Frank et al. 2000, El-Sabah et al. 2011, Sanghvi et al. 2013). Overall, there seems to be general agreement that the insufficient sterilization of the syringes used to administer PAT helped spread these viruses in many schistosome-endemic areas, particularly in Egypt. In addition, frequent blood transfusions, which are associated with hepatosplenic schistosomiasis, appear to have increased the probability of becoming coinfected with HCV in Brazil and perhaps in other geographic areas (see Silva et al 2011).

Once coinfected, however, the clinical course of illness for those with *Schistosoma*-HBV or *Schistosoma*-HCV infections are typically much more severe than for mono-infected subjects. The strongest evidence for this may be inferred from eight prospective cohort studies we reported on in our tables that systematically monitored disease progression in their subjects. Namely, in Table 1.3.3: entry number 10, Bassily et al. 1979; entry number 12, Bassily et al. 1983; In Table 1.3.4: entry number 2, Nooman et al. 1977; entry number 3, Gaffar et al. 1991; In Table 1.3.5: entry number 1, Kamal et al. 2001; entry number 7, Kamal et al. 2006; and in Table 1.4: entry number 3, Kamal et al. 2000; entry number 5, Kamal et al. 2004. The results of these studies are very consistent with one another. With respect to HBV infection, coinfection with *Schistosoma* prolonged the carriage state and more often resulted in chronic hepatitis with greater cirrhosis as well as higher mortality (Bassily et al. 1979, Bassily et al. 1983, Noonman et al. 1977). Much of the same was also observed with respect to HCV, where coinfection with *Schistosoma* was associated with a reduced ability to spontaneously resolve the viral infection and more often resulted in rapid fibrosis as well as higher mortality (Kamal et al. 2000, Kamal et al. 2001, Kamal et al. 2005, Kamal et al. 2006).

The key question is if the effect of coinfection with *Schistosoma* and HBV or HCV is synergistic, i.e., if the combined effect is greater than the sum of each disease in isolation. The best evidence for this may be inferred from the two prospective cohort studies we presented in Table 1.4 (i.e., Kamal et al. 2000 and Kamal et al. 2004), which each compared two mono-infected groups with one coinfected group which were similar with respect to baseline confounding factors. Both of these studies pertain to coinfection with *Schistosoma* and HCV. The earlier of the two studies documented differences in mortality between mono and coinfected groups, while the later study documented differences in the rate of fibrosis. In the first study, mortality among the coinfected (48%) was considerably more than the sum of that observed among mono-infected HCV (12%) or mono-infected *S. mansoni* (3%) subjects during the 72-76 month follow up period of the study (Kamal et al. 2000). More generally, the coinfected patients in this study were characterized by having more advanced liver disease with higher histologic activity and a higher incidence of cirrhosis and hepatocellular carcinoma than either subjects from either mono-infected group. In addition, coinfected subjects also had higher HCV RNA titers with a predominance of HCV genotypes 4 when compared with the mono-infected HCV group.

Similarly, in the later cohort study, the rates of liver fibrosis among the coinfected (0.58 units per year) were again much higher than the sum of those observed for mono-infected HCV (0.1 units per year) or mono-infected *S. mansoni* (less than 0.1 units per year) subjects during the 96 or so

months of follow-up. In both studies, the effect of coinfection appears to be multiplicative rather than additive, supporting the supposition that a synergistic relationship exists between HCV and schistosomiasis. This study also compared HCV-specific intrahepatic and peripheral CD4+ T cell proliferative responses and cytokine production between coinfected and mono-infected HCV patients. At the start of the study, subjects in the HCV infection mono-group had stronger multispecific intrahepatic HCV-specific CD4+ Th1 responses than did coinfected subjects. The coinfected group was characterized as having no T cell responses or weak, narrowly focused responses that over time were maintained only in the liver. In sum, the rate of progression of fibrosis observed in these subjects, as well as HCV virus load, was found to be inversely correlated with intrahepatic HCV-specific CD4+ T cell response. This suggests that the more rapid progression of liver fibrosis is associated with a failure to develop early, multispecific HCV-specific CD4+ Th1 responses in coinfected subjects, and is most likely due to an earlier infection with schistosomiasis that triggered a prior Th2 cytokine response. Numerous studies, all conducted on HCV, generally seem to support the idea of a reduced Th1 host response in coinfected subjects (see Fahmy et al 2006, and more recently Loffredo-Verde et al. 2015). Unfortunately, we lack recent comparative observational studies that would allow us to draw the same level of inference about the effect of coinfection with HBV and schistosomiasis, though the results of the prospective cohort study undertaken by Gaffar et al. 1991 (entry number 3) suggests a certain similarity. For a more detailed discussion on the mechanisms of coinfection between schistosomiasis, hepatitis C and B, we suggest the reader consult the recent reviews by Gasim, Bella and Adam (2015) and Baghat (2014), which summarized the immunological research from a wider range of studies beyond the scope of our analysis; In conjunction with this, these authors also provided a more comprehensive discussion of advances in treatment including antiviral therapy.

In conclusion, the results of our research argue for greater primary prevention for both HBV and HCV in *Schistosoma*-endemic populations. Although no vaccine currently exists for HCV as it does for HBV, additional steps can still be taken to reduce transmission in high risk populations (see Anwar et al. 2008, Lemoine et al. 2013, Lemoine et al. 2014, Vineas and Wild. 2014). Furthermore, vaccination against HBV would also prevent subjects from becoming triple infected, either with *Schistosoma*-HBV-HCV or *Schistosoma*-HBV-HDV, and thus possibly worsening their clinical course as was sometimes documented in a few of our studies (see Zakaria et al. 1993, Zakaria et al. 1994). Additional observational, longitudinal studies conducted on human populations that are fully comparative in nature could help answer some of the remaining questions on both *Schistosoma*-HBV as well as *Schistosoma*-HCV coinfections. Some of these include the role of active vs. past schistosomal infections, the role of genetic variants (see Dessein et al. 2013), as well as the effect of coinfection on treatment. While a thorough discussion of treatment is outside the scope of this review, it has been documented that *Schistosoma*-HCV subjects are less responsive to antiviral therapy than mono-HCV infected subjects (see Abdel-Rahman et al. 2013, El Zayadi 2009); And while *Schistosoma*-HBV coinfected patients may now fare better, additional work on larger populations is still needed (see Huang et al. 2013). Finally, in designing these studies, researchers must also take care to use a sufficient sample size to ensure adequate statistical power, particularly in longitudinal studies where loss-to follow-up is a well-known problem. Surprisingly, only a few studies examined in this review calculated the statistical power needed either in the

1330 design of their study or presented it when evaluating their findings (i.e., Hyams et al. 1986, Kamel
1331 et al. 1994, Al-Freihi 1993).

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