

Population-Based Differences in *Schistosoma mansoni*- and Hepatitis C-Induced Disease

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Two populations with differing histories of *Schistosoma mansoni* and hepatitis C infection were compared directly for severity of disease and extent of comorbidity. Demographic, parasitologic, and ultrasound surveys were conducted on 2038 Egyptians and on 2120 Kenyans. Hepatitis B and C serologies and transaminase levels were obtained from a subset at each site. Despite significantly lower prevalence and intensity of infection, Egyptians had a higher prevalence of severe schistosomal fibrosis than Kenyans (36.8% vs. 4.6%). Hepatitis C infection was 3 times more prevalent among Egyptians, and evidence of hepatocellular damage was significantly greater among Egyptians. There was no interaction between *S. mansoni* infection or disease and the prevalence or severity of hepatitis C. For both infections, the intensity or prevalence of infection was a poor predictor of morbidity. The prevalence of disease in the Egyptian population from different pathogens suggests a generalized susceptibility to inflammatory liver disease.

Marked differences in the severity of disease due to *Schistosoma mansoni* in Africa have been reported [1], but regions rarely have been compared directly. Curiously, some areas with the highest prevalence of hepatic fibrosis by ultrasound, such as the Sudan [2, 3] and Egypt [4], have relatively low levels of transmission. This contrasts with other places, such as Mali [5], Senegal [6], and Kenya (J.H.O., unpublished data), with high transmission but low reported morbidity. The factors responsible for these disparate expressions of disease remain unclear. A few studies have shown a relationship between parasite strain and morbidity in humans [7]. Some of the strongest evidence, however, suggests that host genetic factors are the most important determinant for variation in morbidity [8].

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The ethical review boards of Case Western Reserve University, Cairo University, and the Kenya Medical Research Institute approved all protocols. All participants or their guardians were informed of the potential risks and benefits of the study before providing consent. Human experimentation guidelines of the US Department of Health and Human Services were followed in the conduct of this study.

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Coinfections or other environmental factors might also influence disease expression. Hepatitis C virus (HCV) has been implicated in some studies as a factor influencing the severity of schistosomiasis [9], and, in turn, an influence of schistosomiasis on HCV severity has been suggested [10]. The effects of age, sampling strategies, or the prevalence of exposure to the parasite, however, often confound these studies. Both hepatitis C and schistosomiasis are associated with hepatic inflammation and are prevalent in Africa, but each is characterized by different immunologic mechanisms. Schistosomiasis, like many parasitic diseases, is characterized by predominant Th2-type responses associated with hepatic granulomatous inflammation, whereas Th1-like responses play a role in hepatitis C-induced liver damage and viral clearance [11]. The end result of infection is likewise morphologically and functionally different.

Schistosomiasis produces a distinctive pattern of fibrosis in the liver that follows the distribution of the portal vein branches. This results in portal vein distention and portal hypertension but leaves hepatic function intact [12]. In contrast, hepatitis C is associated with cirrhosis (i.e., destruction of liver cells, disorganization of liver architecture and function, and portal hypertension) and hepatocellular carcinoma. Geographic differences in the distribution of HCV genotypes and response to treatment are well documented [13]. Within populations, differences in morbidity appear to depend primarily on host factors and less on viral genotype [13-15].

Cross-comparison of these 2 diseases in different geographic regions may help identify factors independent of the infecting agents that contribute to morbidity. As part of a study on schisto-

somal hepatic fibrosis, we compared the morbidity due to *S. mansoni* and HCV in communities in Egypt and Kenya.

Materials and Methods

Populations, parasitology, hepatic ultrasound. Between 1999 and 2000, 2 rural communities in endemic areas of Kenya and Egypt were selected for their relatively high prevalence of *S. mansoni* infection. In Kenya, all persons aged > 11 years from the community of Katheka in central Machakos District were examined for schistosome ova in 2 stools and urine [16, 17] and for hepatic fibrosis by ultrasound (*n* = 2120). All participants belonged to the Akamba ethnic group, and demographic data including sex, age, and relationship to head of household were collected by trained local personnel in the local language.

Although an attempt was made to enroll the total willing population in both communities, the population size and distribution in the area defined as the Egyptian community, Shamarka, Kafr el Sheikh governorate, Nile Delta, Egypt, precluded enrollment of everyone. Thus, 40% of the regional population aged > 11 years was examined for schistosome ova in stools and fibrosis by ultrasound, and ~70% of those in the central community and the nearby hamlets (*ezbas*) surveyed (*n* = 2038). As part of a genetic study with an affected sib-pair design, blood was collected from first-degree relatives in families with > 1 sibling with grade C or higher fibrosis. Serologies done at random on the serum samples drawn for these family studies. Prior to the study, there were 3 consecutive years of mass treatment for schoolchildren in this community followed by 3 years of targeted chemotherapy for infected adults and children.

In both communities, we used portable ultrasound units (Shimadzu SDU-350A equipped with 3.5-MHz convex abdominal transducers). One of us (P.M.) reviewed all images from both sites for quality and final determination of pattern. The protocol for ultrasound examination and criteria for evaluation were modified from the most recent World Health Organization report on ultrasound in schistosomiasis [18, 19]. This classification system uses both an image pattern to describe the overall texture of the liver parenchyma as well as measurements of portal vein branch wall thickness and main portal vein diameter measured at standard landmarks. Patterns A and B show normal or mild changes suggestive of fibrosis, respectively. Pattern C includes ring echoes and tubular structures representing the increased fibrosis around portal vessels. Pattern D shows echogenic ruff around the portal stem. Echogenic patches extending from the main portal vein into the parenchyma characterize pattern E, and pattern F has echogenic thickening extending to the surface. Patterns C–F are strongly associated with periportal fibrosis due to schistosomiasis and are not seen in cirrhosis due to viral hepatitis. Image pattern X suggests cirrhosis, and pattern Y suggests fatty liver. Pattern U indicates when the image was not interpretable.

Plasma. In Egypt and Kenya, 10 mL of blood was collected in heparinized tubes from those individuals with significant schistosomal fibrosis and their first-degree relatives. For the Kenyan study population, serology was performed on blood collected from 90% of those with schistosomal fibrosis and their families. In Egypt, blood was collected at 2 different time periods, and serologies were performed on samples obtained during the first collection period, representing

14% of all persons in the study with schistosomal fibrosis and their families. After collection, plasma was separated within 2 h, frozen at – 20° C, and transported on dry ice. Aliquots were thawed only once before serology, transaminase measurement, or RNA extraction. All transaminase measurements and hepatitis serologies were performed at University Hospitals, Cleveland. HCV serologies were determined by HCV third-generation ELISA (Ortho-Clinical Diagnostics). By use of this method, 81% of initially reactive specimens were confirmed as positive using the RIBA assay, 11% were indeterminate, and 8% were negative. All genotyping for HCV was carried out in Brazil, as described elsewhere [20, 21]. In brief, after extraction of RNA from 200 μL of serum, cDNA was synthesized by reverse transcription by using random hexamers. The 5' untranslated region was amplified by polymerase chain reaction (PCR) by use of primers that amplify a 251-nt genotype-specific region. The amplified segment was then subjected to restriction digest and analysis on ethidium bromide–stained agarose gels. Restriction-length polymorphisms in this area define genotypes and the known subtypes [21].

Statistical analysis. The data were analyzed by use of EpiInfo software, version 6.0 (Centers for Disease Control and Prevention). Descriptive statistics, χ² test, 1-way analysis of variance, and linear regression analysis were performed with SPSS statistical software. For linear regression, the image patterns corresponding to normal or schistosomal fibrosis were coded with integers from 0–8, according to increasing severity, by using the published weighting of each

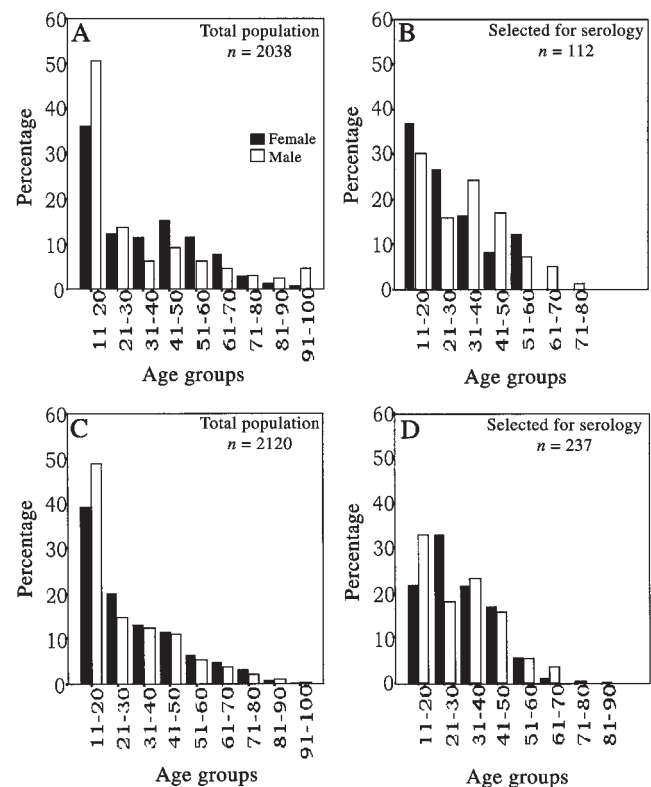


Figure 1. Age and sex distribution for Egyptian (A and B) and Kenyan (C and D) populations studied and for the subsets selected for serologic studies.

Table 1. Population characteristics.

Characteristic	Egypt		Kenya	
	Ultrasound (n = 2038)	Serology (n = 112)	Ultrasound (n = 2120)	Serology (n = 237)
Sex of population, % males	55.8	64.7	42	49.6 ^a
Age, mean years ± SD	31.7 ± 14.0	34.0 ± 15.9	30.7 ± 18.4	33.1 ± 22.2
<i>Schistosoma mansoni</i> prevalence, %	20	23.0	63.6	70.7 ^b
Intensity, mean ± SD ^c	76.9 ± 1.5	69.8 ± 1.5	105.7 ± 3.9	82.9 ± 3.7 ^b
Fibrosis ^d	36.8	48.2	4.6	19.0 ^c

^a Sex ratios are different between Egyptian and Kenyan populations ($P < .05$), by 1-way analysis of variance (ANOVA) comparing differences between populations selected for serologies.

^b $P > .05$ by 1-way ANOVA comparing differences between Egyptian and Kenyan populations for both ultrasound and those selected for serologies.

^c Geometric mean of those infected, expressed as eggs per gram of feces.

^d Percentage of population with hepatic image patterns C–F, excluding patterns suggestive of cirrhosis (X) or fatty liver (Y).

pattern category for comparison with measurements of vessel wall thickness and portal vein diameter.

Results

Population characteristics. In Kenya, all subjects with grade C pathology or higher and their first-degree relatives were selected for serology; in Egypt, blood was collected from 12% of those with significant pathology and their relatives. When we compared the age profiles between sites, the total and the selected Kenyan populations had a steeper age profile than the Egyptian population (figure 1). Males made up 42% of the total Kenyan population and 49.6% of those selected for serology. The total Egyptian population was skewed toward males, and the population selected for serologic testing was significantly more male dominated (54.2% and 64.7%, respectively; $P = .02$, χ^2 test). Both the prevalence and intensity of *S. mansoni* infection (mean egg counts) were higher in Kenyans (table 1).

Ultrasound. Some 70% of Egyptians and 87% of Kenyans tested for serology also had ultrasound examinations. The proportion of the population with hepatic fibrosis was higher for those selected for serology from both sites, and fibrosis was significantly more prevalent among Egyptians (table 1). Because of the selection of affected persons and their families for study, the proportion with significant schistosomal fibrosis (patterns C–F) was greater than for the general population (table 2). For the Kenyans, we reported elsewhere that measurements of portal branch–wall thickness were most associated with the severity of the image pattern used to classify schistosomal fibrosis [19]. For Egyptians, linear regression analysis showed that the portal vein diameter was the best correlate with the ultrasound image pattern ($r^2 = 0.34$, $P = .001$). Of importance, the image patterns indicative of schistosomal fibrosis did not correlate with serologies for viral hepatitis or transaminase levels. Despite the lower prevalence of *S. mansoni* infection, the Egyptian population experienced more schistosomal fibrosis than the Kenyans (table 2). The total Egyptian population likewise had a higher prevalence of other liver abnormalities suggestive of

fatty liver (pattern Y) or cirrhosis (pattern X). There were insufficient subjects with the X image pattern among those selected for serology to determine how well this pattern correlated with cirrhosis due to hepatitis C.

Serology and transaminases. The prevalence of hepatitis B surface antigen was higher in Kenya, but antibody rates did not differ between the 2 sites (table 3). By contrast, the prevalence of HCV antibody–positive subjects was 3-fold higher for Egyptians (39.7% vs. 11.3%). HCV-seropositive Egyptians were significantly younger than HCV-positive Kenyans (mean age, 33.5 ± 14.9 and 39.1 ± 23.7 years, respectively; $P = .005$). Our upper limit of normal for both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) is 67 U/L and can be used as a marker for active disease. By use of multiple statistical approaches, HCV infection was associated with active disease only in the Egyptian population. Mean transaminase levels were significantly higher in HCV-positive compared with HCV-negative Egyptians, but no differences were found for Kenyans on the basis of HCV status (table 4). By using a categorical comparison of subjects above and below the upper limit of normal, there was a significant difference only between seropositive and seroneg-

Table 2. Prevalence of image patterns in Egyptian and Kenyan populations.

Pattern ^a	Egypt, %		Kenya, %	
	All (n = 2038)	Selected (n = 112)	All (n = 2120)	Selected (n = 237)
A	34.2	19.6	95.2	78.5
B	24.6	25.9	2.2	2.5
C	18.1	25.0	1.8	14.8
D	7.0	10.7	0.5	3.4
E	4.8	7.1	0.15	0.8
F	2.9	5.4	0.0	0.0
X	2.5	0.9	0.05	0.0
Y	5.7	5.4	0.1	0.0
U	0.2	0.0	0.0	0.0

^a Image patterns in total population examined and those selected for serologic studies: A, normal; B, suggestive of fibrosis; C–F, increasing severity of fibrosis; U, unknown; X, cirrhosis; Y, fatty liver.

Table 3. Prevalence of viral hepatitis markers.

Marker	Egypt	Kenya
HBsAb	43 (30.5)	109 (38.8)
HBsAg	2 (1.4)	19 (6.9) ^a
HCV	56 (39.7)	31 (11.3) ^a

NOTE. Data are no. (% positive of the population tested). HBsAb, antibody to the hepatitis B surface antigen; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus.

^a*P* > .05 for difference in prevalence between countries, χ^2 statistic.

ative Egyptians (AST, *P* = .011; ALT, *P* = .001, χ^2 test). The relative risk for an elevated transaminase level in HCV-positive Egyptians compared with Kenyans was 5.3 for AST and 1.8 for ALT.

Finally, we used multiple linear regression to determine the relative association between ALT or AST levels and the variables age, sex, log intensity, hepatitis B and C antibody positivity, and ultrasound pattern. For Egyptians, only HCV serology produced a significant correlation for either transaminase (table 5); correlation between ALT and AST levels, respectively, and HCV status were $r^2 = 0.29$ (*P* < .0001) and $r^2 = 0.16$ (*P* < .001). For Kenyans, no variable produced an $r^2 > 0.10$.

Genotype. None of 27 serum samples from HCV-positive Kenyans was typeable, and 85% failed to amplify. In contrast, RNA was amplified from 65% of the HCV-positive Egyptian serum samples, and 54% were typeable. Of the typeable samples, 64% were type 4, 29% were type 5, and 7% were type 1a.

Discussion

By use of the same ultrasound equipment and a single evaluator, we found geographic differences in the severity of schistosomal hepatic fibrosis. An Egyptian living on the Nile Delta was 8 times more likely to have significant fibrosis than a person from central Kenya. This increased risk and severity were independent of the current or past prevalence and intensity of infection in the respective regions. Because the average time from peak infection intensity to hepatic fibrosis is 5–10 years [19, 22], present patterns

of fibrosis reflect infection status of a decade ago. On the Nile Delta, the intensity and prevalence of *S. mansoni* infection is lower than in the Machakos district of Kenya, extending back over decades [19, 23–25]. Factors other than worm burden must explain the variation in schistosomal morbidity.

Some studies suggest that schistosome infection and hepatitis C have a synergistic influence on hepatic morbidity [10]. In this study, however, multivariate regression showed that HCV, hepatitis B, *S. mansoni* infection, and hepatic fibrosis were independent of each other at both study sites. This is consistent with another large community-based study in Egypt [26] and of histopathology of liver biopsies from those infected with both hepatitis C and *S. mansoni* [14]. Therefore, coinfection with the major hepatitis viruses is not a major source of the geographic variation in fibrosis.

The prevalence of both HCV infection and active disease was greater for Egyptians (38.7%) than for Kenyans (11.3%). The third-generation ELISA used was only slightly less sensitive and specific than RIBA or PCR in Africa [27, 28], and the high prevalence in Egypt was consistent with other surveys for the Nile Delta (range, 14%–51% [10, 29]). The few surveys reported from Kenya were from urban blood donors and were lower than we observed in Katheka. We used transaminase levels to assess disease activity and hepatic inflammation. Of all persons infected in either community, only the Egyptians showed a significant association between HCV antibodies and hepatocellular damage. As with schistosomiasis, there were significant geographic differences in both infection and disease due to HCV.

A common cause for differential morbidity in HCV infection is a difference in the age of those affected, which generally reflects the length of exposure to the virus. Here, the mean age of those who were HCV positive was, in fact, higher for Kenyans. Of those who were positive for HCV antibodies, RNA could be amplified from only 15% of the Kenyan samples as compared with 54% of the Egyptian samples. This suggests that the Kenyans more readily obtained self-cures or had lower virus loads. As expected, most of the Egyptian samples were genotype 4. There were a few types 1a and 5, and this may be the first time that these genotypes have

Table 4. Hepatocellular damage and hepatitis C virus (HCV).

Variable	Egypt			Kenya		
	Mean	95% CI	<i>P</i> ^a	Mean	95% CI	<i>P</i> ^a
AST level, U/L						
HCV positive	54.9 ± 29.0	(14.3 to 34.40)	<.001	39.0 ± 13.3	(–7.1 to 6.1)	.882
HCV negative	38.0 ± 6.5			37.4 ± 16.6		
ALT level, U/L						
HCV positive	39.5 ± 29.3	(11.2 to 32.9)	<.001	28.2 ± 17.6	(–3.8 to 6.5)	.593
HCV negative	19.3 ± 8.7			28.4 ± 16.0		
<i>Schistosoma mansoni</i> intensity ^b						
HCV positive	2.9 ± 6.9	(–0.1 to 0.3)	.459	26.7 ± 4.1	(–0.5 to 0.6)	.796
HCV negative	2.6 ± 6.1			27.0 ± 11.0		

NOTE. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; epg, eggs per gram.

^aTwo-tailed *t* test of the difference between HCV positive and negative values.

^bGeometric mean of eggs per gram of feces for egg-negative as well as egg-positive stools.

Table 5. Regression parameters for alanine aminotransferase in the study populations.

Independent variable	Egypt			Kenya		
	r^2	β	P	r^2	β	P
HCV	0.279	0.529	<.001	NE	NE	NE
HBsAb	0.032	-0.106	.044	NE	NE	NE
HBsAg	0.013	0.034	.220	0.009	0.131	.225
Sex	0.010	0.110	.225	0.005	-0.102	.325
Log epg	0.010	-0.064	.250	0.013	0.155	.082
Schistosomal fibrosis	NE	NE	NE	0.099	0.315	<.001
Age	NE	NE	NE	0.040	-0.208	.030

NOTE. ALT, alanine aminotransferase; epg, eggs per gram of feces; HBsAb, antibody to the hepatitis B surface antigen; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; NE, not entered (did not meet criteria for entry into the equation [$P \leq .5$]).

been reported from Egypt. For HCV there has been conflicting evidence as to whether the specific genotype affects the severity of disease [13, 15]. The best indication is for increased morbidity with type 1b [30, 31]. At a minimum, it appears that the influence of these viral genotypes should not account for the sharp differences in morbidity observed here.

Although we have discussed the most likely and the most accessible factors associated with risk of morbidity, we recognize that many other factors could contribute to the observed differences in fibrosis or viral hepatitis. Some studies in laboratory animals suggest that parasite isolates from different geographic regions have differing potential to cause disease [7], but no studies have clearly identified "fibrogenic" strains of *S. mansoni*. While hepatitis B and C were unrelated to schistosomal fibrosis, other coinfecting viruses, malaria, or other parasites differentially infect these populations and could influence disease expression. It is also possible that there is a difference in the use of agricultural chemicals such as organophosphorous pesticides, which are associated with hepatocellular damage and have wide use in Egypt [32, 33], or aflatoxins [34]. Agriculture and diet are substantially different on the Nile Delta compared with the steep hillsides of north-central Machakos.

The ascertainment in Egypt was less complete than in Kenya, but it is unlikely there was significant bias in the ultrasound examinations. The differences in morbidity were so great that had all those not examined had normal hepatic architecture, fibrosis would still be significantly greater in Egypt. Blood was collected from families with increased schistosomal fibrosis in some members, but, since fibrosis was not associated with HCV infection or morbidity, this selection scheme would not also select for increased HCV disease.

The degree to which human genetics is responsible for differential expression of schistosomal fibrosis is currently being pursued. One group identified a locus (SM2) [8] near or at the interferon- γ receptor that was significantly associated with fibrosis, independent of a locus associated with infection intensity (SM1). For schistosomiasis, HLA studies [35, 36] point to multiple alleles associated with increased morbidity. For hepatitis C, there is a

more consistent HLA pattern associated with viral clearance. Eight independent studies indicate that presence of the DQB1*0301 allele is associated with self-cure [37–44]. Population differences in the allele frequency of this class II marker may suggest a mechanism for the differences in the hepatitis C morbidity observed here.

Egyptians show an increased risk for a range of inflammatory liver diseases, compared with Kenyans. Thus, there may be a common pathway for regulation of inflammation in the liver regardless of the initial insult. The findings also mean that the risk for expression of disease cannot be generalized from one population to another on the basis of the presence or prevalence of a pathogen. Specific measurements of morbidity must be made in order to compare the burden of infectious diseases in different communities.

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