Prevalence and Familial Aggregation of Schistosomal Liver Morbidity in Kenya: Evaluation by New Ultrasound Criteria

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Severe periportal fibrosis is not an inevitable consequence of infection with Schistosoma mansoni. Genetic predisposition may be a deciding factor in the development of disease. To assess the contribution of genetic factors in the severity of hepatic fibrosis, the degree of familial aggregation was determined in a Kenyan population. Schistosomal fibrosis was identified with hepatic ultrasound and newly proposed World Health Organization criteria, which include both qualitative and quantitative observations. These 2 aspects of the criteria correlated well with one another. The peak prevalence of ultrasound proven fibrosis trailed 5–10 years behind peak prevalence of infection and declined sharply after age 50 years. This pattern was consistent with either resolution of severe fibrosis over 10–20 years or early death of those severely affected. Genetic predisposition appears to be a weak factor in the development of severe disease in this population, since no household or familial aggregation could be identified.

Infection intensity and disease in schistosomiasis are not strictly related. Neither intensity of infection [1–3] nor prevalence [4] are consistent predictors of disease for individuals or populations. The factors that control infection, therefore, may be significantly different from those that affect pathology. The development of fibrosis may depend on intensity of infection, nutrition, concurrent infection, toxins, or intrinsic characteristics of the host’s response. Differences in parasite strain have been suggested as a source for variation in morbidity, but few studies have tested this as a factor. Human genetics may also play an important role.

Several population-based studies have examined the familial factors related to infection status and intensity [5] but few have examined the factors that control pathology. Evidence for a major genetic contribution to pathology due to Schistosoma mansoni infection was provided by Dessein et al. [6]. By using complex segregation analysis for a Sudanese population, they determined that the distribution of variation in the population was consistent with the effect of a single major gene. A search of candidate regions that included the SM1 locus (5q31-q33) [7], a locus that encodes the interleukin-4 receptor (6q22-q23), HLA–tumor necrosis factor (6p21), and the interferon-γ region (12q15) found that the latter region contained the potential gene. This strongly suggests that human genetic variation may account for a large portion of the heterogeneity observed in this disease in some populations.

The ability to clearly define and measure disease is key to all genetic studies. In addition, methods that quantify disease increase the power of genetic studies. One obstacle to measuring the contribution of various factors associated with pathology has been establishing a standardized, accepted, specific and sensitive method for accurately measuring morbidity due to S. mansoni. Hepatomegaly and intensity of infection have a poor correlation with periportal fibrosis. Even liver biopsy lacks sensitivity, since it is prone to sampling error. At present, ultrasound examination of the liver comes closest to this ideal measure of disease. It is relatively simple to perform and has high community acceptance, a rapid turnaround time for results, and, depending on the criteria, high sensitivity and specificity for histologically proven disease [8–10]. Importantly, at higher grades of disease, ultrasound can distinguish between cirrhosis (destruction of liver architecture) due to viral hepatitis or hepatotoxins from fibrosis due to S. mansoni (periportal distribution of collagen and narrowing of vasculature). A major goal for the use of ultrasound in comparative studies is to establish and validate a common language and common criteria for staging disease. In this study we used a modification of recent World Health Organization (WHO) ultrasound criteria to examine all
members of an *S. mansoni*-endemic community, to assess the relationship of infection intensity, prevalence, and familial aggregation to the development of fibrosis.

**Subjects and Methods**

**Population.** Between October 1998 and January 1999, demographic, stool sample examination, and sonography were done for residents of 4 adjacent villages in Machakos District, Kenya. All persons from the communities of Katheka and Kayatta were included, plus some residents of the adjacent villages of Miseleni and Lower Nduu. Schistosomiasis has been endemic in this area for at least several decades. No major treatment campaigns have been done, except for a 1975 campaign in Lower Nduu [11]. Of an estimated 6000 residents, 4527 were enrolled. Absence because of work in the capital city was the main reason that subjects were not enrolled. The area is ~195.5 km², with steep hills and small seasonal streams and rivers in the valleys. There is one principal permanent river (Kalala) that marks the eastern and northern borders of the study area. Most inhabitants are small-scale produce farmers who work without irrigation and have had family in the area for generations. All participants were of Akamba ethnicity, a group that has occupied the region for more than a century. Demographic data, including sex, age, and relationship to head of household, were collected by trained local personnel, using the local language.

**Parasitology.** Stool samples were collected on 2 different days from all subjects ≥5 years old, and 2 slides were prepared by the Kato-Katz method [12]. Experienced technicians from the Division of Vector Borne Diseases, Nairobi, performed quantitative counts for all helminth eggs. Statistical analysis was done on log-transformed mean egg counts. At the end of the study, all persons infected with *S. mansoni* were treated with praziquantel at a dose of 40 mg/kg of body weight.

To evaluate sites of transmission, 7 river, stream, and dam sites identified by villagers as common use areas were examined for the presence of snails. Each site comprised a 15-m stretch that was searched for 15 min with a standard flat wire scoop (2-mm mesh) mounted on a wooden handle. The collected snails were speciated and were placed individually in flat-bottomed glass vials (7.5 × 2.5 cm in height and diameter) that contained clear water. The vials were placed in soft sunlight to facilitate shedding. The maximum exposure was 4 h. Trained technicians from the Kenya Ministry of Health identified human and nonhuman cercariae.

**Ultrasound.** We used a Shimadzu SDU-350A ultrasound unit with a 3.5-MHz convex abdominal transducer powered by a Honda gasoline generator. One of us (P.M.) reviewed all images and measurements for quality control and final determination of pattern. The protocol for ultrasound examination and evaluation was modified from a draft report of the most recent WHO meeting on ultrasound standards in schistosomiasis [13]. Three elements of this standard were included in the present study: (1) measurement of the subject’s height to the nearest centimeter, (2) identification of the echoic pattern of the liver parenchyma, as developed by J. Richter (Institute of Tropical Medicine, Berlin), and (3) measurement of portal branch wall thickness (PBWT) and portal vein diameter (PVD). Subjects were examined supine, with the transducer positioned to obtain the best image. Liver patterns were designated A, B, C, D, E, F, X, Y, and U, on the basis of the extent of fibrosis or other parenchymal pattern (table 1). Although mixed patterns existed, the most severe pattern was scored for those cases. Persons with any evidence of cirrhosis or fatty liver were considered to be X or Y pattern, regardless of other underlying patterns. Because the consensus view of the WHO working document is that the patterns C–F are primarily due to fibrosis from *S. mansoni* infection, we used these patterns to designate cases. Measurements of portal peripheral branches and portal vein were made only on subjects with these patterns, except for most of those with the B pattern and randomly selected control subjects with pattern A.

For measurements, the external and luminal diameters of the largest first segmental portal branch were measured as closely as possible to the branch point, with magnification to increase accuracy. This segment then was reimaged and measured. We calculated the mean from these 2 measurements, and the inner diameter was subtracted from the outer diameter to yield a value for the 2-wall thickness (PBWT). The outer diameter of the portal vein near its entry into the liver was likewise measured, and the mean of the 2 measurements was calculated (PVD). An index consisting of the mean measurements divided by height was developed to determine whether this was a useful correction for differences in the size of subjects. Ascites, collateral formation, gallbladder thickening, and hepatic masses were recorded, if present.

**Familial aggregation.** Household aggregation of subjects with fibrosis of grade C or greater was examined by comparing the observed aggregation to that expected, assuming a random binomial distribution. Expected numbers were generated by calculating the probability of observing 0, 1, or ≥2 affected household members for each household, on the basis of its size and the population prevalence of C or greater fibrosis. For each class (0, 1, and ≥2), the probabilities of all households were summed to yield an overall expected value for the entire population. We calculated a P value by likelihood ratio χ² statistic, since the sample size was large but the expected values were small. Under these conditions, the likelihood ratio χ² statistic carries less bias than does the Pearson statistic.

The presence or absence of familial aggregation of fibrosis was specifically addressed by examining the relative risk (RR) to siblings, which is a useful statistic for estimating the power of genetic linkage and association studies [14]. In our case, the prevalence of
C+ fibrosis in siblings of affected persons was 0. To examine whether this lack of aggregation was significantly below that expected by random chance, each sibling’s probability of being affected (and of surviving) was estimated by using age- and sex-adjusted fibrosis prevalence. The probabilities then were summed across all 129 siblings, to calculate the expected number of affected siblings. To test whether the observed number of affected siblings differed significantly from the null hypothesis of random aggregation, a random distribution was generated empirically by using the bootstrap technique to simulate the disease of these 129 siblings 30,000 times. For each simulation, the total number of affected siblings was calculated and binned to generate a probability distribution for total siblings affected. An upper confidence interval (CI) for RR was calculated by varying the RR in repeated simulations until the $P$ value became significant (i.e., $P < .05$), which occurred at an RR of 1.1.

Data analysis. Field data were collected on standardized sheets and then were double entered in the Epi Info computer program (version 6; CDC). Egg counts were log transformed to normalize the data. For statistical analysis, Epi Info data were imported into Excel 98 (Microsoft) or SPSS (version 9.0; SPSS) files. We used analysis of variance (ANOVA) to test associations among multiple continuous variables. The ANOVA was adjusted for multiple comparisons by the Bonferroni method. Correlation of continuous variables was determined by the Pearson method. The level of significance for all tests was $<.05$.

Results

Transmission potential. Biomphalaria pfeifferi, found at all sites examined, were the likely principal intermediate host. Snails were found during all study months, except November and December, when floods were caused by brief torrential rains (figure 1). The snail population peaked in March and dwindled from June to August. Infected snails were found throughout the study, except for July and August, when snail counts were very low.

Prevalence and intensity of infection. The age and sex profiles of the region and study population were typical for a developing country. Persons $<19$ years old comprised 48% of the population, and the male:female ratio was near 1.0. The prevalence of S. mansoni infection was 48%, and the highest intensity of infection was 3900 eggs per gram (epg). Six percent of those infected had egg counts $>400$ epg. Age-specific intensity rose sharply to a mean of 40 epg in the 20–29-year-old age group, with a subsequent steep decline (figure 2A). The prevalence of infection rose steeply to a plateau in the 10–19-year-old age group and remained elevated at $\sim 40\%$ until age 60–69 years (figure 2A). There was no significant difference in intensity or prevalence of disease between the sexes: both participate in agricultural activities.

Ultrasound findings. We completed 2115 examinations of all area residents $>12$ years old. We defined fibrosis pattern by new WHO recommendations [13]: 2014 (95%) were pattern A (normal), 46 (2.2%) were pattern B (early suggestive changes), 39 (1.8%) were pattern C (early periportal fibrosis), 10 (0.5%) were pattern D (advanced disease), and 3 (0.1%) were pattern E (severe disease). No person had pattern F (fibrosis extending to the liver surface). One had pattern X (cirrhosis, indicative of hepatitis), and 2 had pattern Y (fatty liver). The peak prevalence of schistosomal fibrosis remained at $\sim 4\%$ for ages 20–49 years, then declined sharply (figure 2B).

Next, we compared measurements of PBWT and PVD from persons with B–E ultrasound pathology with 86 normal liver
patterns selected at random. To determine whether there were meaningful differences between ultrasound pattern categories, mean values were compared by one-way ANOVA for age, height, log egg counts, PVD, and PBWT. There were no differences between ultrasound patterns for age, height, or egg count. Overall, the sex distribution between pathologic and nonpathologic patterns was not statistically different ($P = .06, \chi^2$). The more severe forms of pathology, however, were more prevalent in males. Portal measurements were significantly associated with the liver pattern reading. The more severe the pattern, the more marked the degree of wall thickening and portal dilatation (figure 3). By one-way ANOVA, those with patterns C–E had significantly thicker vessel walls than did those with patterns A or B (table 2). For PVD, pattern E showed a significantly greater mean diameter than did A or B.

During the year-long study, the only 3 people in Katheka who died with a syndrome that included vomiting blood all had C, D, and E pathology. They were 68, 21, and 37 years old, respectively. Neither the liver pattern nor PVD were predictive of imminent death in these subjects.

In some studies, measurements of the portal system depended significantly on the subject’s height [15, 16]. In our study, although height as a continuous variable showed a weak correlation with PVD ($r^2 = .13, P = .006$), the categories for height used in the WHO classification showed no significant differences for PVD or PWBT (table 3). The height-indexed measurements did not change the relationships among the patterns for PBWT. By using the height-indexed PVD, however, the mean for pattern E was significantly greater than for all other patterns. By contrast, the unindexed PVD for patterns C–E were not statistically different.

Aggregation of infection and disease. We compared household aggregation to random aggregation, on the basis of binomial distribution, by using family size and the overall population prevalence of C or greater grade fibrosis. The observed versus expected number of households was 676 versus 674.8 with 0 affected, 46 versus 48.5 with 1 affected, and 3 versus 1.7 with $>2$ affected. In no case did the observed value differ from the expected ($P = .6$).

Familial aggregation was assessed by examining siblings of those affected for evidence of schistosomal fibrosis. There were no sibling pairs observed of 43 affected persons in the community. By using prevalence stratified by age and sex for risk of disease, a random distribution provided an expected mean of 2.7 affected siblings. The 0 observed affected siblings did not differ significantly from the expected by bootstrap simulation ($P = .063; n = 30,000$). In terms of RR, repeated simulations defined a 95% CI of 0–1.1. In essence, the RR is unlikely to be $>1.1$. Overall, sibling aggregation based on prevalence was consistent with a pattern of random aggregation.

Discussion

In Katheka we observed the pattern of infection and disease in a relatively stable community, where $S. mansoni$ infection is endemic, transmission is nearly constant, and no mass chemotherapy has been used in 25 years. The age-intensity and age-prevalence patterns indicate peak intensities occurring in an older age group than in most areas and may suggest that occupational exposure through agriculture may be a significant source of transmission. Against this possibility, the crops and agricultural methods in Katheka do not obviously differ from those in the rest of Machakos District, where intensity peaks at ages 10–19 years (A. E. Butterworth, personal communication). The participation of both men and women in agriculture may also account for the nearly equal distribution of infection or fibrosis by sex. Although the RR for disease (C pattern or higher) in men was slightly higher than that for
Figure 3. Comparison of mean portal branch wall thickness (PBWT; A) and portal vein diameter (PVD; B) to fibrosis pattern. *Measurement significantly greater than mean for A or B fibrosis patterns.

Table 2. Values for portal vein branch wall thickness (PBWT) and portal vein diameter (PVD), by ultrasound pattern.

<table>
<thead>
<tr>
<th>Pattern (n)</th>
<th>Age, years</th>
<th>EPG</th>
<th>Height, cm</th>
<th>PBWT, mm</th>
<th>PBWT/height ×100</th>
<th>PVD, mm</th>
<th>PVD/height ×100</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (86)</td>
<td>34.4 ± 24.1</td>
<td>13.4 ± 10.2</td>
<td>154.6 ± 11.8</td>
<td>3.0 ± 0.7</td>
<td>1.93 ± 0.46</td>
<td>8.6 ± 1.9</td>
<td>5.56 ± 1.20</td>
</tr>
<tr>
<td>B (44)</td>
<td>25.2 ± 14.7</td>
<td>19.6 ± 17.1</td>
<td>154.9 ± 12.1</td>
<td>4.0 ± 1.0</td>
<td>2.57 ± 0.67</td>
<td>7.8 ± 2.4</td>
<td>5.13 ± 1.62</td>
</tr>
<tr>
<td>C (40)</td>
<td>31.7 ± 16.1</td>
<td>8.1 ± 13.0</td>
<td>157.5 ± 12.8</td>
<td>5.3 ± 1.8a</td>
<td>3.37 ± 1.15a</td>
<td>10.4 ± 2.8a</td>
<td>6.63 ± 1.91a</td>
</tr>
<tr>
<td>D (10)</td>
<td>30.3 ± 12.9</td>
<td>37.6 ± 18.3</td>
<td>158.9 ± 10.5</td>
<td>5.8 ± 1.8a</td>
<td>3.70 ± 1.16a</td>
<td>11.7 ± 3.2a</td>
<td>7.40 ± 1.94a</td>
</tr>
<tr>
<td>E (3)</td>
<td>25.0 ± 9.9</td>
<td>33.4 ± 25.5</td>
<td>140.3 ± 18.8</td>
<td>6.5 ± 3.0a</td>
<td>4.61 ± 1.96a</td>
<td>12.9 ± 1.3a</td>
<td>9.40 ± 2.08a</td>
</tr>
</tbody>
</table>

NOTE. Data are mean ± SD. EPG, eggs per gram (geometric mean).a Significantly greater than A and B, by one-way analysis of variance (ANOVA).b Significantly greater than A, B, and C, by one-way ANOVA.

women (RR, 1.61), this did not quite reach statistical significance by \( \chi^2 \) analysis.

There was no association between prevalence or intensity of infection and degree of morbidity, as defined by hepatic periportal fibrosis. Indeed, 23% of those with hepatic fibrosis in this study had no eggs on multiple stool examinations. This may, in part, reflect the delay between infection and the development of disease. The peaks of both intensity and prevalence of infection precede most fibrosis by \( \sim 5–10 \) years. Homaida et al. [17] also found a 10-year interval between peak infection intensity and the development of significant periportal fibrosis. If the discordance between the intensity of infection and the appearance of disease is only due to the timing of examinations, then within the same age group a correlation might still be expected to exist. Those with previously high egg counts should retain elevated counts relative to theiragemates and would be most likely to progress to fibrosis. In Katheka, even within age groups, no such correlation can be demonstrated. Intensity and prevalence of infection, therefore, do not correlate with disease in this community.

The relationship between infection and disease in schistosomiasis has been an area for study since the etiology was first described. In the 1960s, Cheever et al. [18–20], in a series of studies on autopsy specimens and in animals, showed a correlation between intensity of infection and schistosomal fibrosis of the liver. The same association appeared to hold for population studies in which hepatomegaly was the measure of morbidity. However, other population-based ultrasound studies of hepatic fibrosis likewise have not supported any relationship with infection intensity or prevalence [3, 15, 16, 21, 22].

Just as we were unable to correlate fibrosis with prevalence or intensity of infection, neither could we associate fibrosis with host genetic factors. Diseases influenced by a major gene segregating in the population would be predicted to aggregate in households and even more strongly within defined familial groups. Because there are no prospective studies that provide mortality rates for \( S.\ mansoni \)-induced fibrosis in Kenya and because the C–F patterns of fibrosis are largely irreversible [23], aggregation of fibrosis can be modeled just with prevalence rates stratified by age to account for any mortality. In Katheka we were unable to demonstrate familial or household aggregation. Thus, inheritance is a weak factor in the development of fibrosis for this population. This contrasts sharply with findings by Dessein et al. [6], who observed that, for a Sudanese population, schistosomal fibrosis has a genetic predisposition. Ultrasound criteria differ slightly between these 2 studies, but these differences are unlikely to account for the absence of aggregation in our population. The most important difference
probably is the nearly 10-fold higher prevalence of fibrosis in the Sudan. Failure to identify a strong genetic component for fibrosis could be due to the low prevalence of disease in Katheka, resulting in insufficient power to detect differences. In addition, these 2 populations also differ in their experience of schistosome infection. The Sudanese population was recently exposed (1–2 generations), whereas the Kenyan population had been settled in the area for many generations. This opens the possibility of some selection between host and parasite. The demise in 1 year of 3 persons with hepatic fibrosis and esophageal bleeding suggests that schistosomiasis can exert a selective pressure in a population.

The tools used to define disease are a critical factor in looking at infection outcomes. Ultrasound is the best available tool for assessing periportal fibrosis. Ultrasound methods and criteria continue to be refined and to better reflect the nature and degree of *S. mansoni*. The most recent WHO consensus on ultrasound criteria for hepatic fibrosis due to *S. mansoni* [13] includes a reading of the overall pattern of parenchymal fibrosis and measurement of peripheral PBWT and PVD. The ultrasound standard can be applied efficiently when measurements are done solely on the basis of liver pattern (i.e., portal system measurements are made only on those with pathology). On some days, >300 persons were evaluated in Katheka. Measurement of the first hepatic branch rather than the second also saves a significant amount of time necessary to localize these smaller vessels.

Since some quantitative measurements appear to be dependent on the size of the person examined, a nomogram for measurements based on height was developed from a Senegalese population. Although the new guidelines recommend measurement of the first order intrahepatic portal branches, only second order branch measurements are available from a Senegalese reference population [16]. Thus, no comparisons of wall thickness can be made between this Kenyan population and the reference group. However, the mean PVD was very similar for the study population and the WHO reference group. Everyone in our reference population was >12 years old. No differences were seen between height classes for wall thickness, but a few differences were observed for PVD. The use of a height-corrected index normalized the measurement of PVD and brought out some significant differences between ultrasound patterns not seen using the raw data. It is not clear, however, that the measurement of height adds a great deal to the analysis of pathology in adults. Finally, it may not be necessary to always establish local norms, since our independent measurements correlate well with those obtained by others in Senegal.

**Acknowledgments**

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**References**


**Table 3.** Values for portal vein branch wall thickness (PBWT) and portal vein diameter (PVD) for reference population.

<table>
<thead>
<tr>
<th>Height class, cm</th>
<th>No. of subjects</th>
<th>Height, cm</th>
<th>Age, years</th>
<th>PBWT, mm</th>
<th>PVD/height ×100</th>
<th>PVD, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>120–139</td>
<td>10</td>
<td>133.1 ± 5.2</td>
<td>11.9 ± 1.4</td>
<td>2.80 ± 0.67</td>
<td>2.12 ± 0.48</td>
<td>7.98 ± 1.42</td>
</tr>
<tr>
<td>140–159</td>
<td>40</td>
<td>150.1 ± 5.6</td>
<td>27.9 ± 21.0</td>
<td>2.97 ± 0.78</td>
<td>1.97 ± 0.50</td>
<td>7.98 ± 2.04</td>
</tr>
<tr>
<td>160–169</td>
<td>30</td>
<td>164.3 ± 2.8</td>
<td>47.7 ± 24.0</td>
<td>3.02 ± 0.63</td>
<td>1.83 ± 0.37</td>
<td>9.53 ± 1.79</td>
</tr>
<tr>
<td>&gt;169</td>
<td>6</td>
<td>172.3 ± 2.6</td>
<td>48.8 ± 20.4</td>
<td>3.12 ± 0.76</td>
<td>1.80 ± 0.43</td>
<td>9.12 ± 1.82</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean ± SD.

* a Younger than subjects in the 160–169 and >169 height classes, by one-way analysis of variance (ANOVA).

* b PVD significantly less than that of subjects in the 160–169 height class, by one-way ANOVA.