

# Susceptibility to Periportal (Symmers) Fibrosis in Human *Schistosoma mansoni* Infections: Evidence That Intensity and Duration of Infection, Gender, and Inherited Factors Are Critical in Disease Progression

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Lethal disease in *Schistosoma mansoni* infections is mostly due to portal hypertension caused by hepatic periportal fibrosis. To evaluate the factors that may determine severe disease, livers and spleens were examined by ultrasound in a Sudanese population living in a village where *S. mansoni* is endemic. Early (FI), moderate (FII), or advanced (FIII) fibrosis was observed in 58%, 9%, and 3% of the population, respectively. Although FI affected 50%–70% of the children and adolescents, FII prevalence was low in subjects  $\leq 20$  years old but increased sharply (45%–58%) in men 21–30 years old and was associated with the highest infections. Portal and splenic vein diameters were increased in one-third of persons with FII and in almost all with FIII disease. Severe disease, FII or FIII with portal hypertension, affected 6% of the population, was associated with splenomegaly, occurred mostly in adult men, and was clustered in a few pedigrees. These observations suggest that infection intensity and duration, gender-related factors, and inherited factors are important in fibrosis development.

Although schistosomiasis remains a major health problem in countries that rely on extensive irrigation, it is not known why some persons develop severe hepatic disease while others in the same village and living in the same conditions are little affected by the infection. Lethal disease in *Schistosoma mansoni* infections is the consequence of extended fibrosis in the periportal spaces along primary and secondary branches of the portal vein (PV) [1, 2]. Fibrosis is part of the healing process that follows the acute inflammatory granulomatous reaction around schistosome eggs trapped in hepatic small vessels. Advanced hepatic disease in schistosomiasis has been associated with hepatosplenomegaly assessed by clinical examination. Hepatosplenomegaly is more frequent in areas of high transmission and in some studies was associated with the highest infections [3–6]. Hepatosplenomegaly also occurs more frequently in certain families in areas where the disease is endemic [7, 8], and he-

patomegaly with or without splenomegaly has been associated with certain HLA specificities [9–13].

The use of ultrasound has enabled direct evaluation of hepatic fibrosis in large cohorts of subjects. Several criteria have been developed for grading hepatic fibrosis by ultrasound; however, the relationship between the grades has not been fully clarified [14–20]. Ultrasound evaluation has shown that fibrosis is more prevalent than expected among people in areas where schistosomiasis is endemic [18, 19] and that hepatomegaly does not necessarily correspond to the most advanced disease stage [5, 18]. Splenomegaly is a better correlate of severe disease [18], although several authors found no correlation between splenomegaly and grades of fibrosis [21, 22].

The aims of the present study were to examine a population in which schistosomiasis is endemic in order to define the stages in disease progression and to evaluate the role of known epidemiologic and parasitologic factors in the development of hepatic disease. Study subjects lived in a Sudanese village and had never been treated with anti-schistosomal drugs. Fibrosis was evaluated in 792 persons by ultrasound.

## Subjects and Methods

**Study area and study subjects.** This study was done in Al Taw-eel, a village in Gezira State, Sudan, 300 km south of Khartoum and within a 4-h drive of Wad Medani. *S. mansoni* is highly endemic in the Gezira irrigation region. All study subjects were migrants who settled in the village 15–20 years ago; all came from the a region of west Sudan where schistosomiasis is not endemic. The

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people of the village belong to one of two tribes, the Rawashda (33%) or the Tama-Messeria (67%). The village was initially a refugee camp that was built in the middle of the fields where the villagers were employed. The village is built on the banks of a narrow canal that is full for half the year; all houses are clustered within an area  $800 \times 100$  m. Various canals circle the village within a short distance, 10–50 m, of the houses; the only water source is the canal water, which is used for drinking, domestic use (baths, washing), and for field irrigation. Since there is no other water source, all subjects have frequent contact with canal water at several sites. Housing is clustered, so most subjects visit the same water contact sites. Interviews indicated that females reduced their direct contact with the canals after puberty; however, this did not stop them from bathing at home with fresh water from the canal and from entering the canals to fetch water for domestic uses. Agriculture work was associated, especially for adolescent and adult males, with exposure to canal water (field irrigation and bathing after work).

We studied the entire village population (~900) except ~12% who were not available at the time of the first or second echographic evaluations because they were traveling to remote areas. The 792 subjects who had ultrasound evaluations belonged to 65 families.

**Ultrasound evaluation.** Study subjects were evaluated by ultrasound (SSD 500 echo camera and 3.5-MHz convex probe; Aloka, Amsterdam, The Netherlands). Liver size, peripheral portal branches (PPBs), the degree of periportal fibrosis, thickness of PPB walls, spleen size, and splenic vein (SV) diameter were assessed. Livers and spleens were measured as previously described [14, 17–20]. Portal vein (PV) diameter was measured at its entrance to the porta hepatis at the lower end of the caudate lobe on subjects who had fasted ~8–10 h. Thickening of secondary periportal branches was observed for all subjects with FI–FIII disease, and the thickness tended to increase with fibrosis grade. This measurement was less reliable than that for PVs and SVs because it depended largely on the PPB stretch that was selected for the measurement; therefore, we did not use this measurement.

We graded periportal fibrosis as grades 0–III. Grade 0 corresponds to normal liver with no thickening of the PPB wall and PPB diameters (outer to outer) ~2–3 mm. Grade I corresponds to a pattern of small stretches of fibrosis around secondary portal branches (this patchy fibrosis usually has a “fishes in the pond” appearance) and PPB diameters ~4 mm. Grade II still shows the patchy fibrosis observed in FI, but a continuous fibrosis affects most second-order branches, and PPBs appear as long segments of fibrosis. PPB diameter is ~5–6 mm. In <30% of cases, the thickness of the gallbladder wall is increased (2–4 mm). Grade III shows a thickening of the walls of most PPBs, some branches are occluded, and the long segment of the fibrosis reaches the surface of the liver. The thickness of the gallbladder wall is usually >4 mm.

We computed the index liver size (ILS) by the following formula [23]:  $ILS = (PSL^2 + MCL^2 + AAC^2) \times 0.2618$ , where PSL is parasternal length, MCL is the didclavicular length, and AAL is the anterior axillary length. The spleen volume was computed as  $(\text{spleen length} \times \text{width} \times \text{depth}) \times 0.523$ .

**Parasitologic procedures and treatment.** Eggs were counted by using Kato's method [24] on  $\geq 4$  stools collected on different days. A small fraction of the subjects also excreted eggs of *Schistosoma haematobium* in urine. All subjects were treated with praziquantel

within 1 year of the start of the study. When necessary, treatment was repeated once to improve the cure as assessed by 3 stool examinations 2–3 months after treatment. *Plasmodium falciparum* infections were also monitored by blood smears, which showed that malaria was endemic in the village.

**Statistical analysis.** Categorical data were compared by  $\chi^2$  test. Comparison of quantitative data was done by analysis of variance on log-transformed data. When geometric means were used, the means are given  $\pm$  the largest of the two errors.

## Results

**Prevalence of fibrosis grades by age and gender.** Fibrosis grades were evaluated with ultrasound in 792 subjects. Thirty percent (30.4%) of the study population had normal livers with no evidence of fibrosis (F0); most subjects (58.2%) had mild fibrosis (FI). Grades II (FII) and III (FIII) were observed in 11.3% of the subjects. FII, observed in 9.2% of the population, was 4.5 times more frequent than FIII (2.1%). Gender had a marked effect ( $\chi^2$ , 36.8, 3 df;  $P < .0001$ ) on the prevalence of advanced fibrosis since in males, prevalences of FII and FIII were 14.5% and 4.1%, respectively, while in females they were 4.7% and 0.5%. Fibrosis grades also depended on age (figure 1): among children 6–10 years old, FI was detected in 62%; among young male subjects (<21 years old), the prevalences of FII and FIII were 3.3% and 0.46%, respectively; among adults (>20 years old), they were 30.4% and 9.3%. An unexpected finding was the high prevalence (45% and 58%) of FII among men 21–25 and 26–30 years old. The peak of FII prevalence among men coincided with a decrease in the prevalence of FI (figure 1), indicating that FII probably developed from FI. Among women 20–30 years old, there was only a small increase in FII prevalence and no decrease in FI prevalence.

**Advanced fibrosis grades are associated with increases in PV and SV diameters.** We attempted to characterize liver and spleen disease by measuring PV and SV diameters and liver and spleen volume. PV and SV diameters, index liver size, and spleen volume were not significantly different in F0 and FI subjects of all ages, indicating that these measurements were normal in subjects with FI. In subjects <16 years old, whether FII or FIII, these measurements were also nearly normal. However, in older people with FII and FIII disease, average PV and SV diameters were increased. Most subjects with FIII and a few with FII exhibited marked splenomegaly; the size of the left liver lobe in general was decreased in these subjects. PV and SV diameters and liver and spleen volumes are shown by grades in figure 2 for subjects >16 years old. These graphs confirm that PV and SV diameters and spleen volume correlate strongly with fibrosis grades.

**Grade 2 fibrosis (FII) phenotype is heterogeneous.** Although all FIII subjects but 1 had PV diameters above the normal threshold (defined as the average PV value plus 2 SD in F0 subjects in the same age class) and evidence of varices, only 35.6% of FII subjects had abnormal PV diameters. This

**Table 1.** Evidence for two clinical subgroups in boys  $\geq 15$  years old with grade FII fibrosis compared with values for patients with other grades of disease.

Variable	F0 or FI	FII		FIII
		PV <14 mm	PV >14 mm	
No. of subjects	256	43	21	17
PV diameter (mm)	11.9 (0.1)	12.9 (0.2)	15.3 (0.2)	15.1 (0.3)
SV diameter (mm)	7.6 (0.1)	8.3 (0.3)	10.4 (0.6)	11.0 (0.5)
Liver (mm <sup>3</sup> )	114.4 (1.7)	106.7 (4.2)	111.7 (5.3)	92.2 (4.2)
Spleen (mm <sup>3</sup> )	247 (11.3)	269.2 (21)	513.7 (99.1)	617.8 (112.9)

NOTE. F0, no fibrosis; FI, early fibrosis; FII, moderate fibrosis; FIII, advanced fibrosis; PV, portal vein; SV, splenic vein. Values for liver and spleen are volumes. Data are geometric mean (SE).

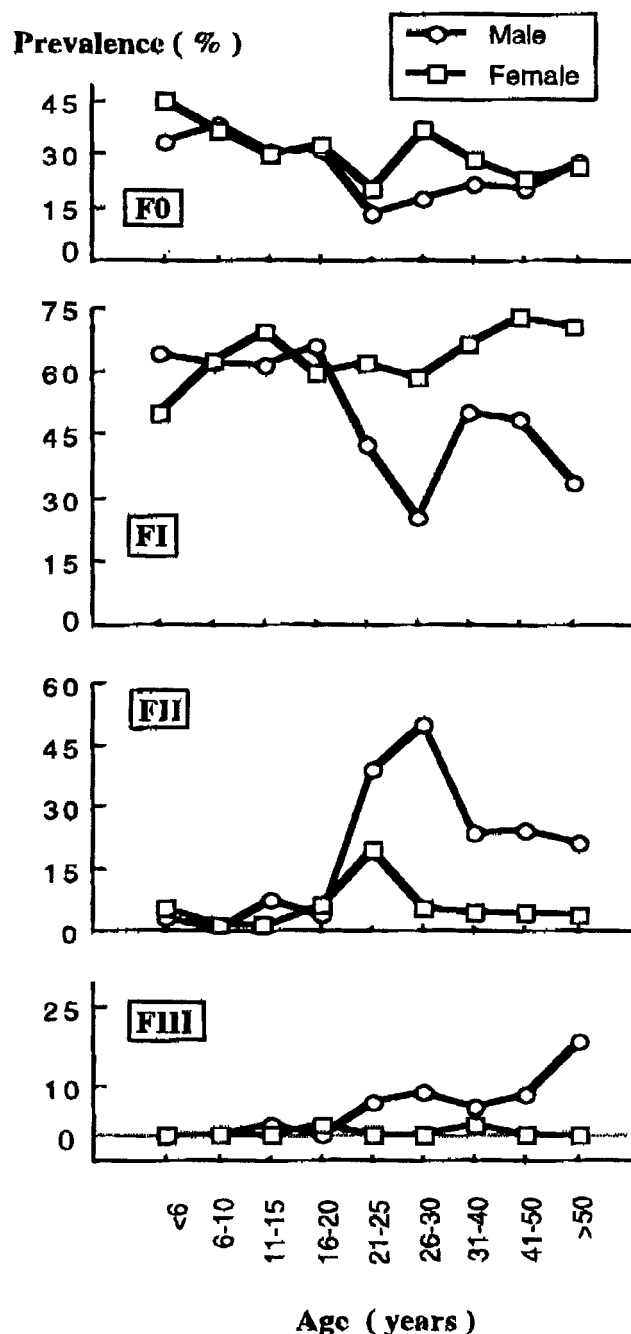
indicates that the FII group included persons at different stages of disease development: some had evidence of portal hypertension but others did not. This conclusion was also supported by measurements of SV diameter and spleen volume that were on average significantly higher in the FII group with abnormal PVs than in the FII group with normal PVs (table 1). These results indicate that among subjects affected by FII fibrosis, some have more advanced disease than others. Thus FII is not a homogenous clinical phenotype but can be divided in two subtypes on the basis of ultrasound observations.

*Duration and intensity of infection are risk factors in advanced fibrosis.* The prevalence of infection in the Al Taweel population was 72.8% in males and 68.3% in females. In subjects <20 years old, the prevalence was comparable ( $P = .3$ ) in males (71.6%) and females (75.6%). Above 20 years, the prevalence of infection was significantly higher ( $P = .001$ ) in males (75.2%) than in females (46.7%). This difference was mostly due to a drop of prevalence among women aged 21–30 years (figure 3), from 85% among girls aged 11–15 to 38% in women aged 26–30. In contrast, prevalence among males remained high until age 40, reaching 90% in those 21–25 and then beginning to decrease in the oldest subjects. A remarkable finding is the parallel between the marked decrease in infection prevalence in women and the low prevalence of FII in women aged 21–25 and 26–30; in contrast, the proportion of FII sharply increased to 45% and 58% in men aged 21–25 and 26–30 years (figure 3).

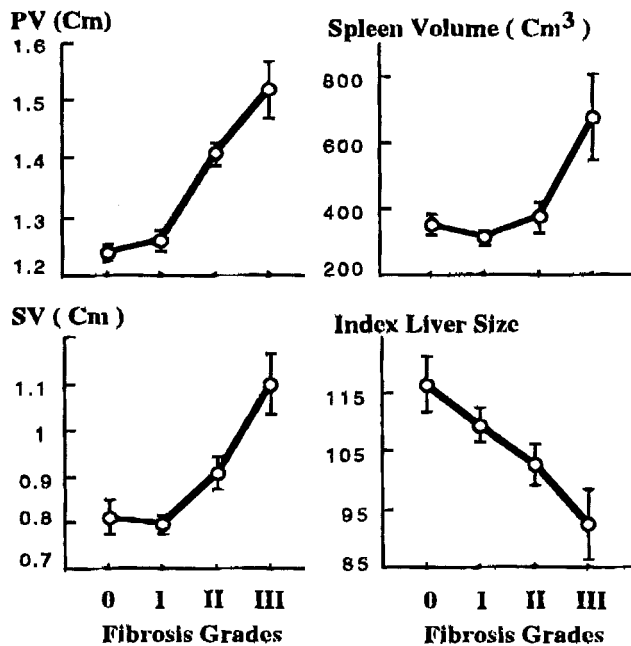
Despite the high prevalence of schistosome infections, the intensities of infection were not elevated (figure 4). Egg counts were the highest in adolescents, with an additional peak for men between ages 30 and 40 years. Infection levels were on average higher in girls than in boys <20 years old ( $P = .03$ ); above age 20, men were more infected than women ( $P < .01$ ). In all age classes, differences in infection levels (in infected subjects) between the sexes were not dramatic. Nevertheless, there was an association between FII and the highest infection levels in males ( $P < .05$ ; figure 5). No such association was found in females or in FIII subjects.

*Clustering of FII and FIII by pedigree.* Analysis of the

distribution of FII and FIII by pedigree showed that these grades of infection were more prevalent in certain pedigrees than in the whole population: 57% of males >19 years old with FIII fibrosis belonged to 4 pedigrees that represented only



**Figure 1.** Prevalence of fibrosis by age and gender in the study population. F0, no fibrosis; FI, early fibrosis; FII, moderate fibrosis; FIII, advanced fibrosis as defined in Methods.



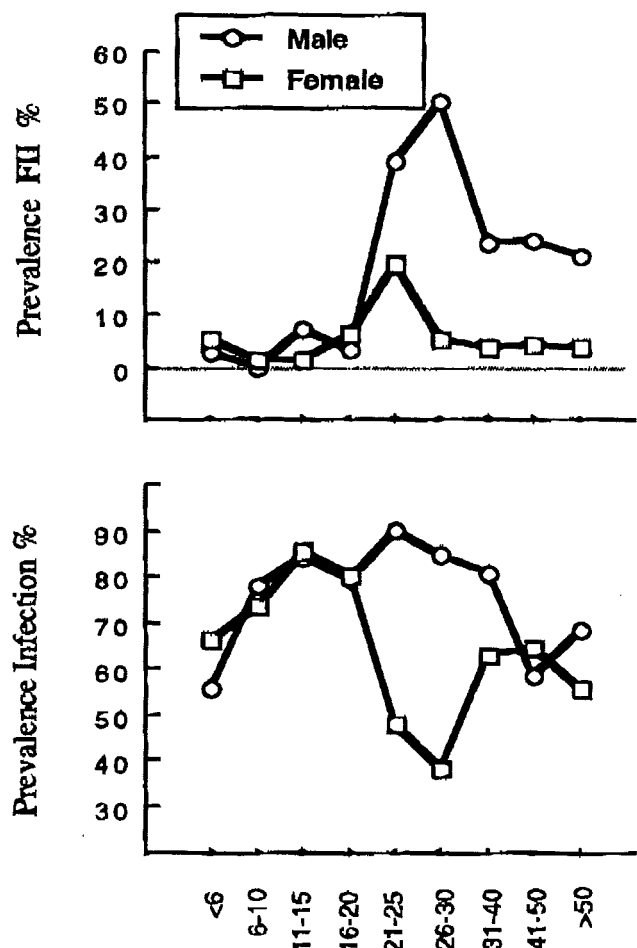
**Figure 2.** Moderate (FII) and advanced (FIII) fibrosis are associated with increased portal vein (PV) and splenic vein (SV) diameters and with splenomegaly. Normal PV and SV diameters and spleen volume depend on gender and increase with age in subjects <16 years old; for this reason, data are presented for male subjects >15 years old (80% of FII and FIII cases are in men >21 years old). Results are arithmetic means; vertical bars, SEs. Analysis of variance showed significant association between grades and PV and SV diameters and spleen volume ( $P < .001$  for male and female subjects for all 3 comparisons) and for grade and liver size index (male subjects,  $P = .017$ ; female subjects,  $P = .09$ ).

15.8% of the adult males in the village. Likewise, 71.4% of women >19 years old with FII fibrosis (FIII seldom occurred in females) belonged to 6 pedigrees that represented only 17.4% of the adult females. These observations (shown in figure 6) indicate a concentration of moderate and advanced fibrosis in certain pedigrees that cannot be explained as an effect of age or gender. The ratio of FII to FIII was also highly variable among pedigrees. Although FII was observed in 53.8% of the adults belonging to pedigree 7 ( $n = 13$  adults) and 27.3% of those belonging to pedigree 5 ( $n = 22$ ), no FIII was observed in these pedigrees. Conversely, although 17.6% of adults belonging to pedigree 22 ( $n = 24$ ) were affected by FIII, no FII was found in this pedigree. Thus disease progression from FII to FIII might not occur at the same rate in all pedigrees.

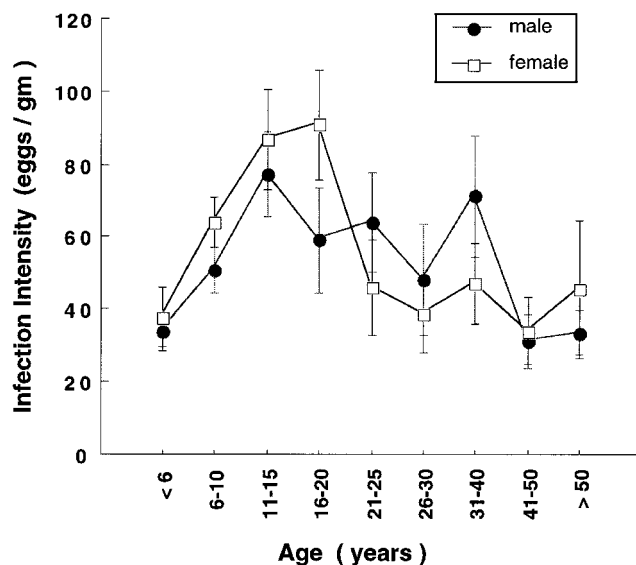
There was no statistically significant differences between members of the Rawashda and Tama-Messeria tribes when clinical and parasitologic data were analyzed. There was, however, a trend for more severe disease (larger PV diameter, larger spleen) in the Rawashda tribe (data not shown).

## Discussion

The aims of the present work were to characterize the principal stages of development of hepatic disease in a Sudanese population of an area endemic for *S. mansoni* and to attempt to identify some of the most critical factors in disease development. The population selected for this study has been living for 15–20 years in a village of the Gezira region, which is well known to be endemic for *S. mansoni* [25]. The village had never been included in a mass treatment program, and the large majority of study subjects had never been treated with schistosomicidal drugs. Two of us previously analyzed risk factors for high infections by *S. mansoni* in a Brazilian population [26–28]. It would have been logical to analyze the risk factors for fibrosis in the same population, but this was not possible because repeated treatments for >15 years have modified the expression of the fibrosis phenotypes in that population, reducing the prev-



**Figure 3.** Low prevalence of moderate fibrosis (FII) in women is associated with a marked reduction in prevalence of schistosome infection.



**Figure 4.** Intensity of schistosome infection in infected subjects by age and gender. Results are geometric means (SEM).

absence of advanced grades [29] and modifying the relative effects of the different risk factors.

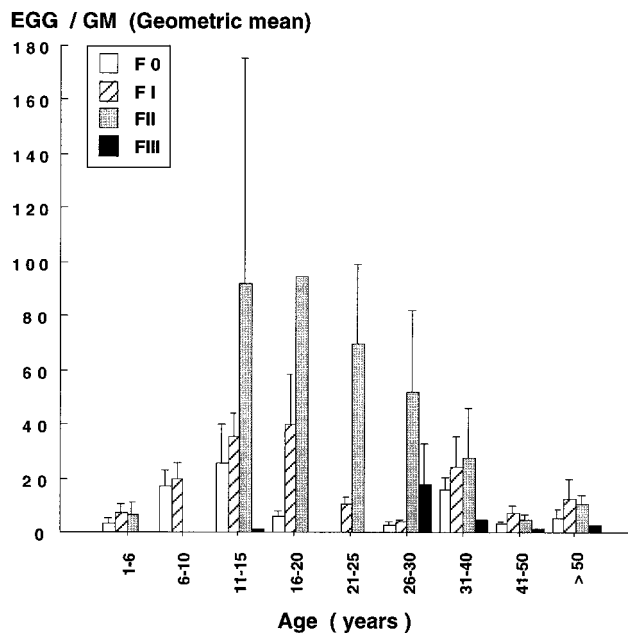
Prevalence of *S. mansoni* infection assessed on 4 or 5 stool examinations was ~70%, which probably indicates, given the sensitivity of five Kato examinations, that 80%–90% of the population harbored living infections. In contrast to the high prevalence, infection intensities were not high; most subjects excreted <100 eggs/g (geometric mean, 34 and 38 eggs/g in infected males and females, respectively). The prevalence of infection was high (>80%) and stable in males 11–40 years old, and the intensity of infection in infected subjects did not vary markedly in this age group, suggesting that strong acquired anti-infection immunity has not developed yet in males of the village. The observation that prevalence and intensity of infection decrease sharply in females aged 15–30 years might have at least two explanations: a reduction of water contact after puberty in young girls and/or some hormonal effects on immunity occurring at puberty or associated with pregnancies.

**Ultrasound evaluation of hepatic disease.** Ultrasound is a valuable, noninvasive tool for evaluating fibrosis [14–19]. Our study also showed that qualitative grading of fibrosis by the Cairo scale [20] can be associated with quantitative measurements (PV, SV, and spleen volume) to define the most advanced disease stages. Subjects with FII disease could be assigned to two subgroups, one with and the other without evidence of portal hypertension. Portal hypertension in these FII subjects was indicated by abnormal PV [30, 31] and SV diameters. These subjects also exhibited enlarged spleens, as did FIII subjects (table 1). These findings are consistent with observations in

previous studies that FII and FIII correlate with varices and with bleeding from esophageal varices [16, 21, 31].

The strong association between splenomegaly and the most advanced fibrosis grades was unexpected in a population that was also infected by *P. falciparum*. Thus, splenomegaly in that population is mostly caused by schistosome infections, as has been reported in some other [18] but not all populations [22, 31], which indicates that splenomegaly has different etiologic origins depending on the population. Our present work and those of others (reviewed in [5]) [22, 31] have failed to associate hepatomegaly with advanced fibrosis. On the contrary, the size of the liver, particularly the right lobe, decreased with increasing fibrosis grades, probably because of the shrinkage of fibrotic tissue.

**Staging of disease from mild to severe fibrosis.** The data presented here help clarify the relationship between fibrosis grades [18]. They indicate that disease likely progresses from FI to FII before reaching the most advanced FIII stage. This conclusion is supported first by the chronology of the appearance of FI, FII, and FIII: FI appears first in the young pop-



**Figure 5.** Moderate fibrosis (FII) is associated with highest infection levels in male subjects. Infection intensities are shown for infected subjects only. Analysis of  $\log_{10}(1 + \text{eggs/g})$  shows significant difference ( $P < .05$ ) between groups of subjects with no fibrosis (F0), early fibrosis (FI), and FII. When data from uninfected subjects are included, differences increase between F0 and FII and between FI and FII groups; it does not increase the significance of the test ( $P < .05$ ). No significant differences in infection levels were observed between F0 and FI, F0 and advanced fibrosis (FIII), or FI and FIII in male subjects and between any fibrosis groups in female subjects. The difference between F0 and FI in male subjects 16–20 years old is not statistically significant.

ulation, FII is rare in children but frequent in young adults, and FIII begins to occur in adults. Second, the sharp increase of FII prevalence associated with an equivalent reduction of FI prevalence in 20- to 30-year-old men suggests that FII developed in subjects that had FI. Third, subjects with FII can be distinguished into two subgroups, one (67% of the FII subjects) with PV and SV diameters and spleen volumes comparable to those of FI subjects, and the other with measurements comparable to those in persons with FIII. This supports the view that FII is intermediate between FI and FIII.

*Some host-specific factors control the development of FI.* FI was detected early in life: 63% and 50% of girls and boys, respectively, aged 1–6 years exhibited FI, and during adolescence the proportion of subjects with FI was 59%–69% for both sexes. The reasons why some children and adolescents do not develop detectable FI cannot be differences in the prevalence of infection: in children 1–5 years old, the prevalence of infection was 45% for subjects with F0 and 55% for subjects with FI; in adolescents, the prevalence of infection was 87% (F0) and 93% (FI). The reasons also cannot be either differences in the intensities of infection, which were comparable for F0 and FI in children (figure 5); a gender effect (the proportions of F0 in boys and in girls <16 years old were the same); or different living conditions (also similar in these study subjects). This suggests that some unidentified host-specific factors may play a critical role in the development of FI. Nevertheless, although moderate and advanced fibrosis grades correlate well with the degree of fibrosis observed in biopsies [19], no liver biopsies have been performed in subjects with FI, probably because such patients are not hospitalized. Thus it remains unclear whether FI represents true early fibrosis.

*Gender and intensity and duration of infection in disease progression from FI to FII.* Three observations indicate that gender and the level and duration of infection play a role in the progression of fibrosis toward FII. First, except for a few persons, FII developed in the third decade of life, suggesting that long-standing infections are required for advanced fibrosis. Second, the low prevalence of FII in women aged 20–30 compared with men of the same age was associated with a drop in the prevalence of infection in young women. Third, FII was associated with the highest infections in males. Nevertheless, infection levels are comparable in 16–30 year old infected males and females, and it is possible that the low prevalence of FII in females is not entirely explained by the 50% decrease in prevalence of infection. Thus the possibility should be considered that young adult males may be more prone to FII than females of the same age. This difference in the prevalence of infection between males and females has been reported in other studies in Egypt in a Muslim village population and in Brazil [32, 33]. In both cases, the researchers reported that differences in exposure to water canals could not fully account for this gender effect; thus, it was suggested that females develop better resistance to infection than males. Finally, growth deficits as-

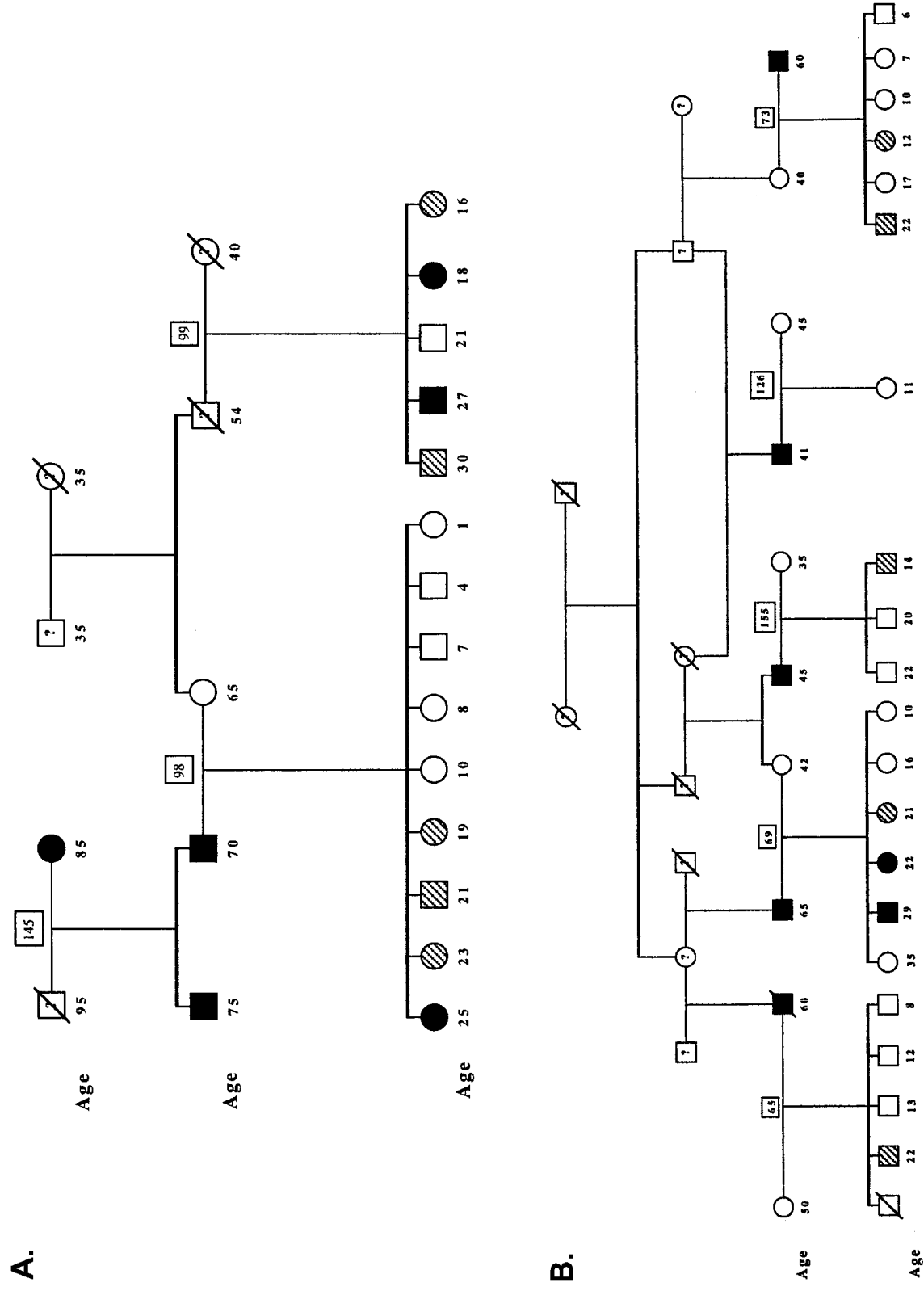
sociated with *S. mansoni* infections have been shown to be more severe in boys than in girls [34].

The positive association between infection intensities and advanced fibrosis has not been found in all studies [5], possibly because FIII is not associated with the highest infections, as discussed below, or because the level of infection is not the only critical factor in severe fibrosis and might be hidden by genetic or other factors. FII prevalence peaks sharply in men 21–30 years old. These subjects had had FI for 15–20 years, and no apparent dramatic changes in infection (prevalence or intensity) were observed in males aged 21–30 or 16–20 years. This finding suggests that some factors that cause disease progression may only appear in late adolescence or in young adulthood. This, together with the indication that young males might be more prone to FII than females, suggests that some gender-specific factors may play a significant role in FII development at the end of the second decade of life. Good candidates for such role are androgens [35, 36], which decrease the level of interferon (IFN)- $\gamma$ , the strongest antifibrogenic cytokine in the granuloma [37–39]. Of interest, estrogens have the opposite effect; they increase IFN- $\gamma$  gene transcription [35, 36]. In conclusion, the data support the view that intensity and duration of infection and gender-related factors may be important in disease progression from FI to FII.

*Inherited factors in disease progression toward FIII and portal hypertension.* FII progressed to FIII only in a small fraction of FII subjects, indicating that some factors may also be important in disease progression at this stage. No evidence was obtained that FIII developed in the most-infected subjects, indicating that egg load is probably not critical for progression from FII to FIII, although it is important for FII development from FI. It has also been suggested that advanced disease may interfere with egg excretion [40]. The observation that FII and FIII cluster in certain pedigrees cannot be accounted for by differences in age, gender, or location (nuclear families are located in various parts of the village), which suggests that some inherited factors may be important in the progression of fibrosis from FII to FIII. Testing the statistical significance of this family clustering is a complex analysis that must take into account several covariates and the parent-parent and parent-offspring relationships. Such an analysis [41] confirms the clustering of FII and FIII in certain families.

The present study failed to reveal clear differences in disease development and in infection between the two ethnic groups in the village. This does not necessarily mean that such differences do not exist. The Rawashda group is too small to do multivariate analysis on FII and FIII with several covariates (e.g., age and gender), and the statistical tests may fail to reach significance because of the small size of the subgroups. Nevertheless, there was a trend for more severe disease in the Rawashda.

An important observation in this study is that progression from FII to severe disease with hypertension is not just the



**Figure 6.** Moderate (FII) and advanced (FIII) fibrosis are clustered in certain pedigrees. Two pedigrees, Tama Messeria (A) and Rawashda (B), are shown. Closed symbols, subjects with FIII; shaded symbols, subjects with FII; open symbols, subjects with no fibrosis (F0) or early fibrosis (FI). Nos. under symbols are ages (in years); household nos. are in squares.  $\square$ , male subjects;  $\square$ , female subjects. Slash indicates subject who died before start of study.

result of a regular and linear evolution of the disease with time but that it probably requires additional factors and that inherited factors are likely to play an important role. We have reported that infection intensities are controlled by a major gene [27, 28, 42, 43]. It has also been reported that hepatomegaly associated with or without splenomegaly in *S. mansoni*-infected subjects is influenced by HLA [9–13]. We tested whether SM1 or HLA could account for the familial clustering of advanced fibrosis; our ongoing study indicates that severe fibrosis is under the control of a genetic locus that is linked neither to SM1 nor to the HLA locus [41].

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