Original Research Article

Genetic and Other Factors Controlling the Effect of Praziquantel on Liver Fibrosis in *Schistosoma mansoni* Infected Patients

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Abstract

To evaluate the genetic and other factors controlling the effect of Praziquantel (PZQ) therapy on the regression of liver fibrosis in an endemic population. Periportal fibrosis (PPF) in one hundred seventy-seven Sudanese patients infected with *Schistosoma mansoni* [82 (46%) males and 95 (54%) females] was evaluated by ultrasound between 1999-2002. DNA from patients infected with *S. mansoni* was extracted using standard salt-putting method, purified and amplified by PCR. Allelic typing was done using RFLP. SPSS (Statistical Package for Social Science) software was used for statistical analysis. Chi-Square was used to compare the two phenotypes (regression and progression) in the study subjects. Regression phenotype was reported in 63 (35.6%) patients, while the disease progressed to higher grades of fibrosis in 24 (13.6 %) patients. No change in grade of fibrosis in 90 (50.8%) patients. The mean values of spleen volume (S.Vol.) in patients with regression phenotype were found to be significant when compared to that in patients with progression one (*P*=0.003). Progression phenotype in males (15, 8.5 %) was greater than that of females (9, 5.1 %). Patients with regression phenotype were clustered in certain families. The possible genetic control of PPF has been evaluated by studying the role of polymorphism (IFN-γ rs2069705 (C/T) in the regression of PPF. No significant association between the polymorphism (IFN-γ rs2069705 (C/T) and PPF as response to PZQ (*P* = 0.9). Our study indicated that PZQ therapy has a great effect on regression and stabilization of PPF. The regression of liver fibrosis was controlled by gender, age, grade of fibrosis, and possibly inherited factors.

Key words: Periportal fibrosis (PPF), Polymorphisms, Praziquantel (PZQ), Progression, Regression

INTRODUCTION

Schistosomiasis is a chronic and debilitating, and remains one of the most prevalent parasitic infections in tropical and subtropical environments (WHO, 1993). Genetic factors explain, at least in part, why some individuals resist infection in general more successfully than others do, although they are living in the same environment with the same living conditions. Other factors such as health condition, acquired immunity and the variability of infectious agent have contributory effect (Kwiatkowski, 2000).

In human *Schistosomiasis* many reports mentioned the antifibrogenic effect of interferon-γ (IFN-γ) in hepatic fibrosis (Duncan and Berman, 1985, Tamai et al., 1995, Mallat et al., 1995 and Marquet et al., 1999). Recent
studies had shown that human susceptibility to *S. mansoni* infection is controlled by genetic loci: *SM1* located in chromosome 5q31-q33 which controls the infection levels in Brazilian population (Dessein *et al.*, 1999b) and we have shown that susceptibility to PPF is controlled by *SM2* which located in chromosome 6q22-q23 and that is closely linked to *IFNGR1* (gene encoding the alpha chain of the IFN-γ receptor) in a Sudanese population (Henri *et al.*, 2002). Polymorphisms such as *IFN-γ* +2109 and *IFN-γ* +3810 were associated with severe hepatic fibrosis in human (Chevillard *et al.*, 2003). Factors such as gender, age, duration and intensity of infection were reported to have an impact on regression of liver fibrosis (Mohamed-Ali *et al.*, 1999). We have shown in the same cohort of patients that severe PPF is associated with an increase in *TNF-α* production and the progression to severe PPF in *Schistosomiasis* was not associated with polymorphisms in the *TNF-α* gene (Moukoko *et al.*, 2003).

In the present study, we aim to evaluate the genetic and other factors controlling the effect of praziquantel (PZQ) therapy on the regression of liver fibrosis in an endemic population.

### METHODS

This study was carried out between 1999 and 2002 in Um-Zukra village, Gezira state, Managil province, Central Sudan. A medical history, personal data (name, sex, age and number of pregnancies for married women), current symptoms, number of malaria attacks per year and physical examination for each subject were performed.

Informed consent was obtained from each patient or parents in the case of children. Parasitological examination was done using Kato's method (Katz *et al.*, 1972). All subjects were treated with Praziquantel tablets (40 mg kg/ body weight), manufactured by Medochemie Ltd, Limassol, Cyprus, Lot No. E5K020). Ultrasound evaluation was performed as described before (Rahoud *et al.*, 2010). DNA from patients infected with *S. mansoni* was extracted using standard salting-out method (Sambrook *et al.*, 1989), purified and amplified by PCR (Model MBS 0.25 Hybaid,) in a 30μl reaction. PCR conditions were initial denaturation step at 94 °C for 5 min, second denaturation 35 cycles at 94 °C for 1 min, annealing temperature at 53 °C for 45 seconds, first elongation at 72 °C for 45 seconds and final elongation at 72 °C for 10 min. Polymorphism (*IFN-γ* rs2069705 (C/T)) was identified using RFLP method.

SPSS (Statistical Package for Social Science) software was used for statistical analysis and Chi-Square was used to compare the two phenotypes (regression and progression) in the study subjects.

PCR fragment of 303bp length including the *IFN-γ* rs2069705 polymorphism was generated with use of a forward primer (Seq.# rs2069705 F) TCCAATGTGC-CAAAATAATAAAAA and a reverse one (Seq.# rs2069705 R) AAGCCCTCACTTTTGGTT (All form Operon Biotechnologies, GmbH, 50829, Germany). The PCR product was digested overnight at 37 °C in water path with restriction enzyme AluI (Roche Diagnostics GmbH, Mannheim, Germany). The digested PCR product (5 μl) was mixed with 5 μl Loading dye (Bromophenole blue) in acrylamide gel, run in TBE 1% at 110 volts for 2 hours, stained with ethidium bromide (0.5 μg/ml) for 10 minutes, and then visualized under the photo-documentation system (Baby Imager, Appligene).

The *Alu*I analysis of the PCR product obtained from subjects bearing the *IFN-γ* rs2069705 homozygous T/T alleles gives three bands (193, 110 and 19 bp) on acrylamide gel (Sigma). Subjects bearing the *IFN-γ* rs2069705 homozygous C/C alleles also give three bands (174, 110 and 19 bp). Whereas the same analysis performed on subjects bearing the *IFN-γ* rs2069705, heterozygous T/C alleles give four bands (193, 174, 110 and 19 bp). Bands 110 and 19 bp are not relevant in the genotyping (Figure 8).

### RESULTS

The study was conducted in an endemic area for *S. mansoni* in central Sudan. Fibrosis grades were reported before and 39 months after treatment with PZQ (Figure 1 and 2). Patients with FI, FII and FIII before therapy were (128, 72.3%), (29, 16.4%) and (20, 13.3 %) respectively (Figure 1). Thirty-nine months after treatment with PZQ, there was inter-movement of fibrosis grades. Patients with grade (FI), 34.4% of them were reversed to grade (F0), 15.6% were remain stable (no change in fibrosis grade), 11.7% were progressed to grade (FII) and 2.3% were progressed to grade (FIII). Patients with grade (FII), 17.2% of them were reversed to grade (F0), 27.6% were reversed to grade (FI), and 34.5% were remain stable while 20.7% were progressed to grade (FIII). In patients with grade (FIII) there was no regression to grades (F0) and (FI) while 30% of them were regressed to grade (FII) and 70% remain stable (Figure 2).

When the phenotypes were reported as percentages, 35.6% were regression, 50.8% were stable and 13.3% were progression (Figure 3).

Figure 4 shows the clustering of regression phenotype in certain families indicating the possible involvement of inherited factor in the proses of regression and reversibility of periportal fibrosis after PZQ therapy.

As shown in figure 5, regression and stability of PPF phenotypes were more likely in patients of younger age (< 20 years) while progression phenotype was more frequent in older patients (> 20 years) *P* = 0.065. No significant difference was observed in regression of PPF between males (30, 17%) and females (33, 18.6%) with *P*=0169. However, there is more progression of PPF in males (15, 8.5%) compare to females (9, 5.1%). The high
Figure 1. The fibrosis grades before treatment with PZQ tablets (40mg/kg body weight)

Figure 2. The fibrosis grades after treatment with PZQ tablets (40mg/kg body weight)

Figure 3. State of PPF 39 months post-treatment reported as phenotypes
Figure 4. The clustering of regression phenotype (Half-dark symbols) in certain families, stable phenotype (Crossed symbols) and not evaluated person (Open symbols).

Figure 5. Age group and PPF after treatment

Figure 6. Evidence for gender effect on the development of the disease after treatment with Praziquantel (40 mg/kg body weight).
number of females with stable PPF (53, 29.9%) was greater than the number of males (37, 20.9%). This indicates that PZQ stabilizes PPF more in females (Figure 6).

The mean values of spleen volume (S.Vol.) in patients with regression phenotype were found to be significant when compared to that in patients with progression ones ($P=0.003$, Figure 7).

Figure 8 and Table 1) show the genotyping and allele frequency of polymorphism IFN-$\gamma$ rs2069705 (C/T) in patients infected with S. mansoni. No difference in genotype frequency of C/C homozygous in patients with regression phenotype and those with progression phenotype (1, 0.74 %) in both cases. The genotype frequency of C/T heterozygous and T/T homozygous together was (46, 33.8 %) in patients with regression.

Figure 7. S.Vol. (Mean) in regression and progression groups

Figure 8. Genotyping of IFN-\textgamma rs2069705 polymorphism (C/T) in DNA samples of Sudanese patients infected with S. mansoni showing T/T, C/C homozygous and T/C heterozygous using RFLP method.
phenotype and was (88, 64.7 %) in those with progression phenotype. No association was reported between IFN-γ rs2069705 (C/T) and disease prognosis ($P = 0.5$).

### DISCUSSION

The main objective of the present study is to evaluate the genetic and other factors controlling the effect of Praziquantel (PZQ) therapy on the regression of liver fibrosis in a population endemic with *Schistosoma mansoni* infection.

Our study shows a significant regression of PPF 39 months after PZQ therapy which is consistent with the previous studies (Mohamed-Ali et al., 1991, Doehring-Scherdtfeger et al., 1990 and Homeida et al., 1996) however, in this study we were able to report a higher degree of reversibility of PPF (63, 35.6%, Figure 3).

The mechanism of PZQ treatment seems to decreases the infection level by killing the parasites, decreases the number of eggs trapped in the hepatic tissue, and this leads to decrease in granuloma formation, which in turn decreases the fibrogenesis (Utzinger et al., 2000, Homeida et al., 1991 and Garba et al., 2001). PZQ prevent the formation of extra fibrous tissue. It was not known whether PZQ have an effect on existing fibrosis (Fibrolysis), but it is possible to activate the metalloproteinase enzyme, which degrades the fibrosis tissue.

Age and grade of fibrosis both are associated with regression of PPF. The fact that low fibrosis grades are responsive to PZQ treatment could be due to the nature of the content of the fibrosis tissue. Early stages of PPF are more reversible after PZQ treatment (Homeida et al., 1991).

Our findings indicated that PPF has progressed more in males (15, 8.5%) than in females (9, 5.1%, Figure 6). Females seem to benefit from PZQ treatment more than males and respond much better than males to PZQ treatment. Experimental animal studies support our observations (Cavalcanti et al., 2002). Previous studies reported that female reproductive hormones have an antifibrogenic effects (Xu et al., 2002), while male reproductive hormones have a fibrogenic effect (Colborn et al., 1993).

Many parasites cause chronic infections in human with mild clinical symptoms, while others cause severe disease (Dessein et al., 2001). Genetic factors explain, at least in part, why some individuals resist infection in general more successfully than others do, although they are living in the same environment with the same living conditions. Other factors such as health condition, acquired immunity and the variability of infectious agent have contributory effect (Kwiatkowski, 2000).

In the present study, our findings showed that the disease in some patients (13.6%) progressed from lower grades of fibrosis to higher ones following PZQ therapy (Figure 2 and 3). This finding was consistent with the finding of Li et al., (2002) who reported progression of the disease in some patients in a cohort of 120 individuals in China. The explanation for this phenomenon was that either those patients were genetically susceptible to develop severe PPF and that fibrosis once started, progresses in spite of PZQ therapy or they did not respond adequately to treatment or the combination of both effects.

The large number of patients in the present study (50.8%) in whom PPF was stable (no change in fibrosis grades before and after treatment) does not mean that the pathology of the disease had stopped, but we think that those patients may need more time after treatment (> 39 months) in order either the disease reverse or may progress; or the praziquantel therapy should be repeated as reported previously (Kheir et al., 2000, Wynn et al., 1998). However, PZQ was able to stabilize the disease.

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In the present study, we were able to report regression of splenomegaly (S Vol. = 184.4 ± 22.4) compared to those in whom PPF was progressed (S Vol. = 264.6 ± 47.5, Figure 7). This finding was inconsistent with Doehrig-

### Table 1. The frequency of different genotypes of IFN-γ rs2069705 (C/T) when cross-tabulated with the disease prognosis.

<table>
<thead>
<tr>
<th>Disease prognosis</th>
<th>C/C (%)</th>
<th>C/T or T/T (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1 (0.74)</td>
<td>46 (33.8)</td>
<td>47 (34.6)</td>
</tr>
<tr>
<td>Progression</td>
<td>1 (0.74)</td>
<td>88 (64.7)</td>
<td>89 (65.4)</td>
</tr>
<tr>
<td>Total</td>
<td>2 (1.5 )</td>
<td>134 (98.5)</td>
<td>136 (100)</td>
</tr>
</tbody>
</table>

$P = 0.5$
Schwerdtfeger’s finding (Doehring-Schwerdtfeger et al., 1990), who reported regression of hepatomegaly but not splenomegaly in patients 23 months after PZQ therapy. However, our results were consistent with other investigators who reported regression of splenomegaly two years after either praziquantel or Oxamniquine therapy (Kilpatrick et al., 1981, Sleight et al., 1985). Regression phenotype was found to be clustered in certain families. This observation may indicate the possible involvement of inherited factors in the regression of PPF.

In human schistosomiasis many reports mentioned the antifibrogenic effect of interferon-γ (IFN-γ) in hepatic fibrosis (Duncan and Berman, 1985, Tamai et al., 1995, Mallat et al., 1995 and Marquet et al., 1999). Recent studies had shown that human susceptibility to S. mansoni infection is controlled by genetic loci: SM1 located in chromosome 5q31-q33 which controls the infection levels in Brazilian population (Dessein et al., 1999b) and we have shown that susceptibility to PPF is controlled by SM2 which located in chromosome 6q22-q23 and that is closely linked to IFNGR1 (gene encoding the alpha chain of the IFN-γ receptor) in a Sudanese population (Henri et al., 2002).

In addition to other factors which include gender, age, duration and intensity of infection (Mohamed-Ali et al., 1999) we have shown in the same cohort of patients that severe PPF is associated with an increase in TNF-α production and the progression to severe PPF in Schistosomiasis was not associated with polymorphisms in the TNF-α gene (Moukoko et al., 2003). It has also been reported that hepatomegaly associated with or without splenomegaly in patients with S. mansoni infection is influenced by HLA (Baza and Asser, 1985, Secor et al., 1996). SM2 locus was found to be neither linked to SM1 nor to the HLA locus (Dessein et al., 1999b).

CONCLUSION

The study provides strong evidence for substantial regression and stabilization of PPF after PZQ therapy. Regression of liver fibrosis after PZQ therapy is controlled by gender, age, grade of fibrosis and possibly inherited factors.

Although there is no association was reported between IFN-γ rs2069705 (C/T) and disease prognosis, the role of inherited factor in the regression of liver fibrosis is not excluded. Further studies are needed in this regards.

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