MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Microbiology and Biological Sciences (ISSN: 2408-7076) Vol. 3(2) pp. 012-019, November, 2015
Available online http://www.meritresearchjournals.org/mbs/index.htm
Copyright © 2015 Merit Research Journals

Original Research Article

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

[*1]Siddig A. Rahoud (PhD), [2]Adil Mergani (MD, PhD), [3]Awad A. Algarni (PhD) and [4]Nasr Eldin M. A. Elwali (PhD)

[1]Associate Prof., Parasitology and Molecular Biology, Albaha University, Faculty of Science and Arts, Baljurashi, KSA

[2]Associate Prof., Molecular Biology, College of Applied Medical Sciences – Tarabah, Taif University, KSA

[3]Assistant Prof., Genetics, Albaha University, Faculty of Science and Arts, Baljurashi, KSA

[4]Professor, Biochemistry, Department of Biochemistry, College of Medicine, Al Imam Muhammad Ibn Saud Islamic University, KSA

***Corresponding Author's Email: srahoud@gmail.com**
Tel: +966 530215649

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 salting-out 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 = 0.5*). 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-CAAAATAATAATAAA and a reverse one (Seq.# rs2069705 R) AAGCCCTCCACTCTTTGGTT (All form Operon Biotechnologies, GmbH, 50829, Germany). The PCR product was digested overnight at 37 °C in water path with restriction enzyme *Alu*I (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 *Alu1* 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

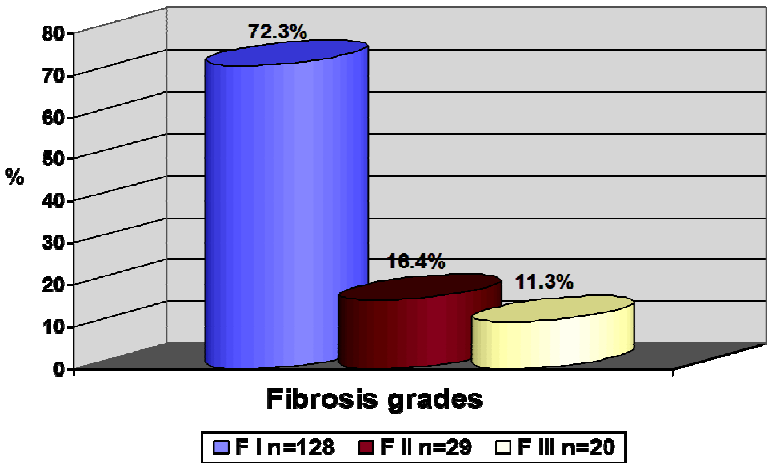

Figure 1. The fibrosis grades before treatment with PZQ tablets (40mg/kg body weight)

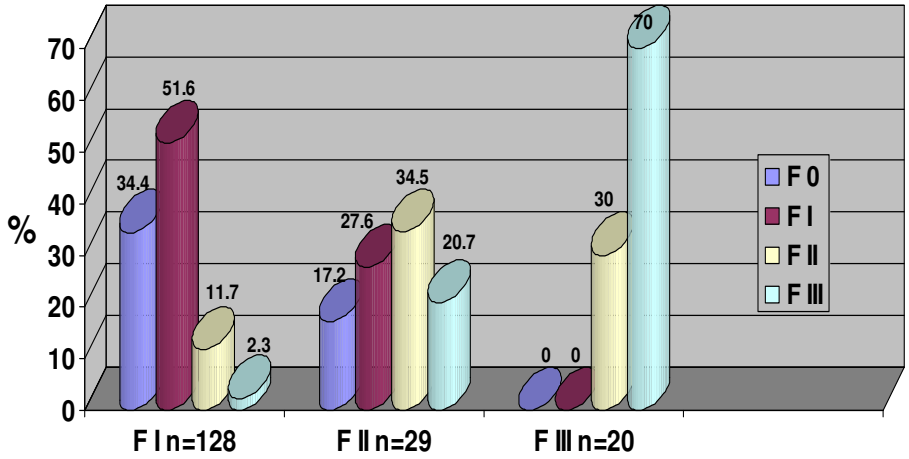

Figure 2. The fibrosis grades afetr treatment with PZQ tablets (40mg/kg body weight)

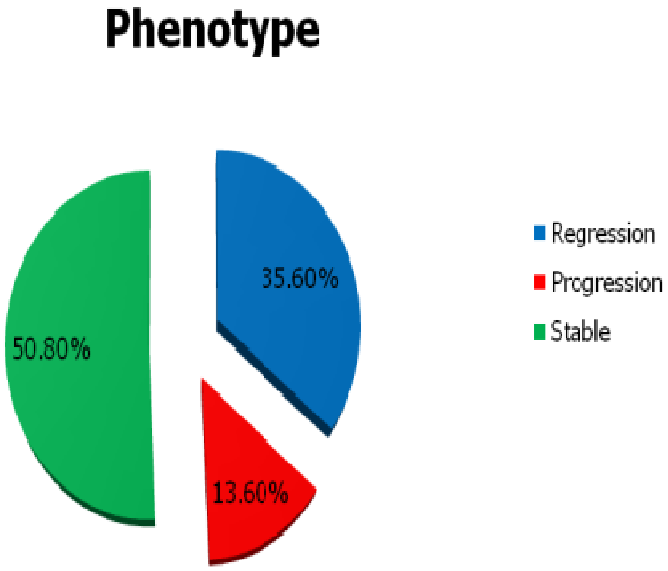

Figure 3. State of PPF 39 months post- treatment reported as phenotypes

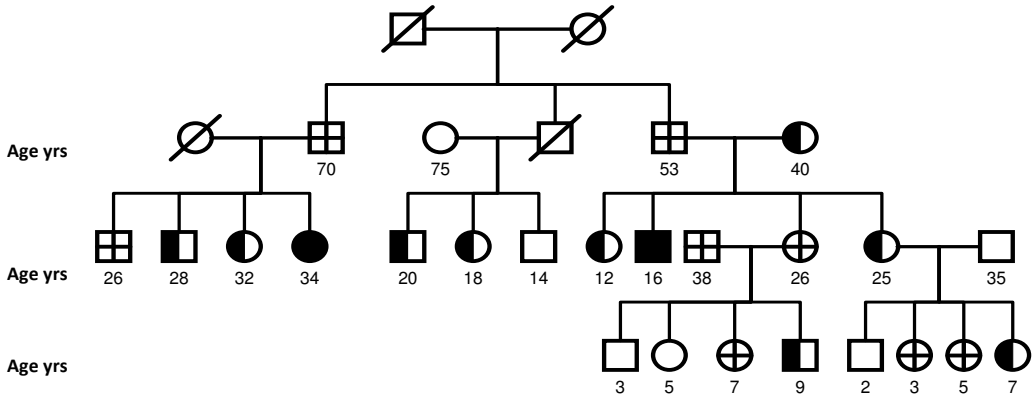

Figure 4. The clustering of regression phenotype (Half-dark symbols) in certain families, stable phenotype (Crossed symbols) and not evaluated person (Open symbols).

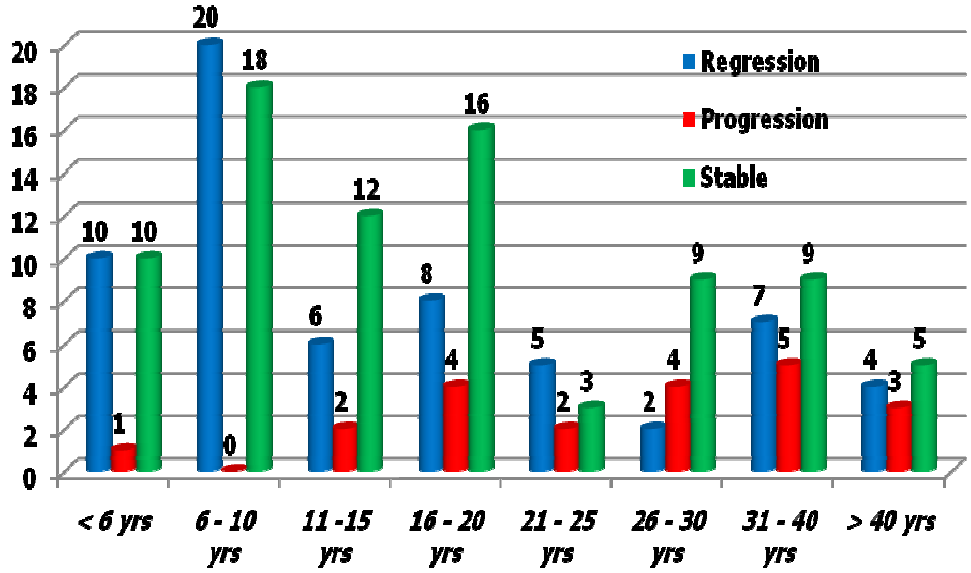

Figure 5. Age group and PPF after treatment

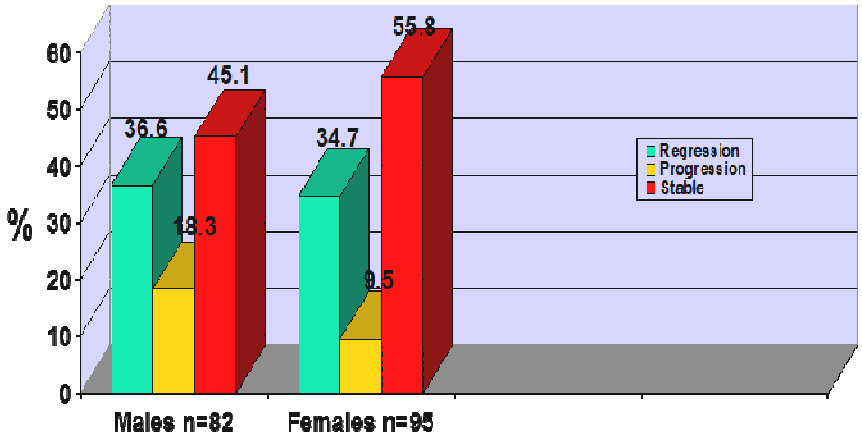

Figure 6. Evidence for gender effect on the development of the disease after treatment with Praziquantel (40 mg/kg body weight).

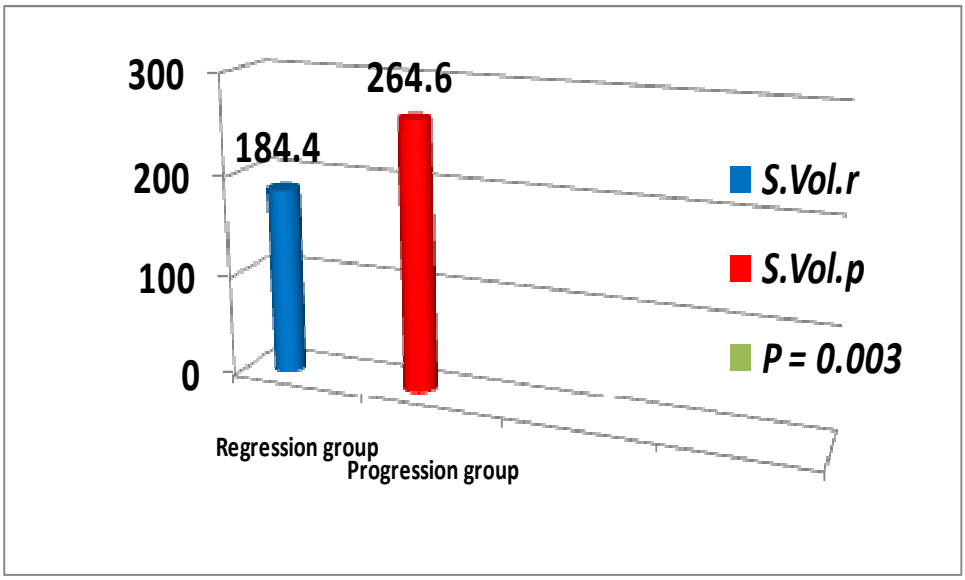

Figure 7. S.Vol. (Mean) in regression and progression groups

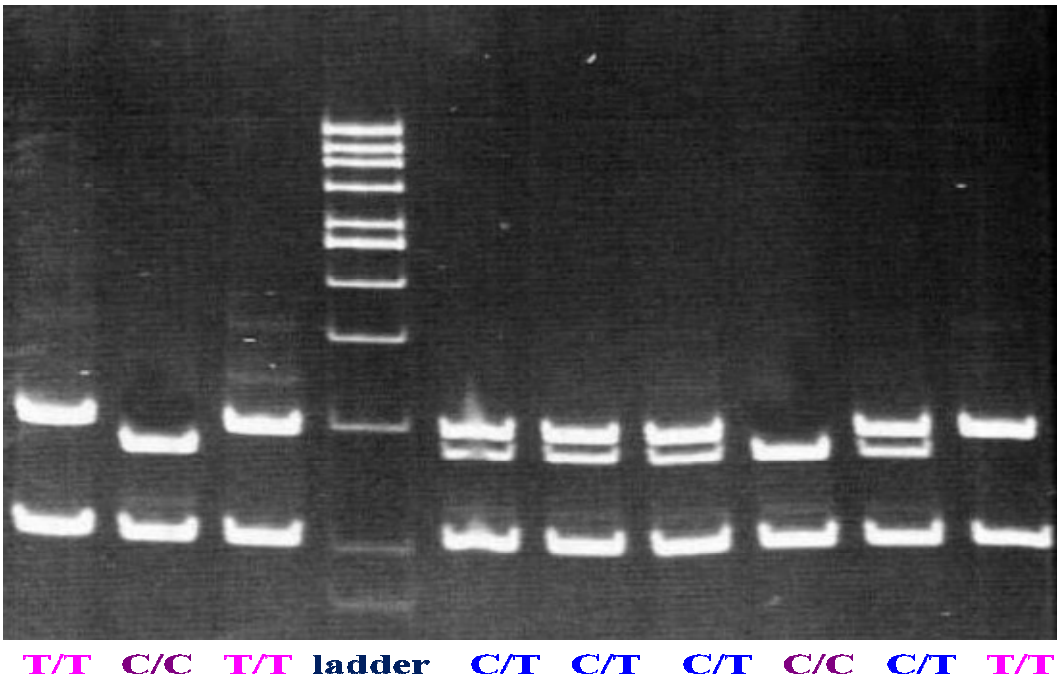

Figure 8. Genotyping of IFN-γ rs2069705 polymorphism (C/T) in DNA samples of Sudanese patients infected with S. mansoni showing T/T, C/C homozygous and T/C heterozygous using RFLP method.

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-γ 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

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

Disease prognosis	C/C (%)	C/T or T/T (%)	Total
Regression	1 (0.74)	46 (33.8)	47 (34.6)
Progression	1 (0.74)	88 (64.7)	89 (65.4)
Total	2 (1.5)	134 (98.5)	136 (100)

P= 0.5

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-Schwerdtfeger *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 seams 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 seam 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. 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-

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, Sleigh *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.

ACKNOWLEDGMENT

The authors would like to thank the people of Um-Zukra village for their continuous cooperation. This study was supported by the Institute of Nuclear Medicine, Molecular Biology and Oncology, University of Gezira, Sudan. Also our thanks to Ministry of Higher Education and Scientific Research for their partial financially support.

REFERENCES

Baza H, Asser L (1985). HLA antigens in schistosomal hepatic fibrosis with haematemesis. *Tissue Antigens* 26: 307-309.

Cavalcanti A, Barbosa JR, Arvon A, Andrade ZA (2002). Contribution to the study of collagen degradation. *J. Bras. Patol. Med. Lab*. 38:(4) 325-332.

Chevillard C, Moukoko CE, Elwali NMA, Bream JH, Kouriba B, Argiro L, Rahoud S, Mergani A, Henri S, Gaudart J, Mohamed-Ali Q, Young HA, Dessei AJ (2003). IFN-γ polymorphisms (IFN-γ +2109 and IFN-γ +3810) are associated with severe hepatic fibrosis in human hepatic schistosomiasis (Schistosoma mansoni). *J. Immunol*. 171: 5596-5601.

Colborn T, vom SFS, Soto AM (1993). Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ Health Perspect*. 101::378–384.

Dessein AJ, Chevillard C, Marquet S, Henri S, Hillaire D, Dessein H (2001). Genetics of parasitic infections. *Am. Soc. Pharmacol. Exper. Therap*. 29:484-488.

Dessein AJ, Marquet S, Henri S, El Wali NMA, Hillaire D, Rodrigues V, Prata A, Mohamed Ali Q, Gharib B, Reggi M, Magzoub MMA, Saeed OK, Abdelhameed AA, Abel L (1999b). Infection and disease in human S. mansoni are under distinct major gene control. *Microbes Infect* 1: 561–567.

Doehring-Schwerdtfeger E, Abdel-Rahim IMA, Mohamed-Ali Q, Elsheikh M, Schlake J, Kardroff R, Franke D, Kiaser CH & Ehrich JHH (1990). Ultrasonographical investigation of periportal fibrosis in children with Schistosoma mansoni infection: evaluation of morbidity. *Am J Trop Med Hyg* 42:581–586.

Duncan MR, Berman B (1985). Gamma interferon is the lymphokine and beta interferon the monokine responsible for inhibition of fibroblast collagen production and late but not early fibroblast proliferation. *J Exp Med* 162: 516–527.

Duncan MR, Berman B (1985). Gamma interferon is the lymphokine and beta interferon the monokine responsible for inhibition of fibroblast collagen production and late but not early fibroblast proliferation. *J. Exp. Med*. 162: 516-527.

Henri S, Dessei AJ, Chevillard C, Paris P, Godard J, Camilla C, Saeed OK, Mirgani A, Bucheton B, Rahoud S, Elwali NMA, Fert V, Montero F, Mohamed-Ali Q, Magzoub MMA (2002). Cytokine regulation of periportal fibrosis in humans infected with Schistosoma mansoni: IFN-gamma is associated with protection against fibrosis and TNF-alpha with aggravation of disease. *J Immunol* 169: 929–936.

Homeida MA, El Tom IA, Nash T, Bennett JL (1991). Association of the therapeutic activity of praziquantel with the reversal of Symmers fibrosis induced by Schistosoma mansoni. *Am J Trop Med Hyg* 45: 360–365.

Homeida MA, Eltoum IA, Ali MM, Suliaman SM, Elobied EA, Mansour M, Saad AM, Bennett JL (1996). The effectiveness of annual versus biennial mass hemotherapy in reducing mmorbidity due to schistosomiasis: a prospective study in Gezira-Managil, Sudan. *Am J Trop Med Hyg* 54: 140–145.

Katz N, Chaves A, Pellegrino J (1972). A simple device for quantitative stool thick smear technique in Schistosoma mansoni. *Rev I Med Trop* 14: 397–400.

Kheir MM, Baraka OZ, El Tom IA, Mukhtar MM, Homieda MMA (2000). Effects of single-dose praziquantel on morbidity and mortality resulting from intestinal schistosomiasis. *EMHJ* 6: 926–931.

Kilpatrick ME, Farid Z, Bassily S, El-Masry NA, Trabolsi B, Watten RH (1981). Treatment of schistosomiasis with Oxamniquine. *Am J Trop Med Hyg* 30: 1219–1222.

Kwiatkowski D (2000) Science, medicine, and the future. *BMJ* 321: 1061–1065.

Li YS, Sleigh AC, Tanner M, Dessein A, Li Y, Williams GM, McManus

DP (2002). Five-year impact of repeated praziquantel chemotherapy on sub-clinical morbidity due to Schistosoma japonicum in China. *T Roy Soc Trop Med H* 96:438–443.

Mallat A, Preaux AM, Blazejewski S, Rosenbaum J, Dhumeaux D, Mavier P (1995). Interferon alpha and gamma inhibit proliferation and collagen synthesis of human Ito cells in culture. *Hepatology* 21: 1003–1010.

Marquet S, Abel L, Hillaire D and Dessein AJ (1999). Full results of the genome-wide scan which localises a locus controlling the intensity of infection by Schistosoma mansoni on chromosome 5q31-q33. *Eur J Hum Genet* 7: 88–97.

Mohamed-Ali Q, Doehring-Schwerdtfeger E, Abdel-Rahim IM, Schlake J, Kardorff R, Franke D, Kaiser C, Elsheikh M, Abdalla M, Schafer P, Ehrich JHH (1991). Ultrasonographical investigation of periportal fibrosis in children with Schistosoma mansoni infection: reversibility of morbidity seven months after treatment with praziquantel. *Am J Trop Med Hyg* 44: 444–451.

Mohamed-Ali Q, Elwali NMA, Abdelhameed AA, Mergani A, Rahoud S, Elagib K, Saeed OK, Abel L, Magzoub MMA, Dessein AJ (1999). 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. *J. Infec. Dis*. 180: 1298-1306.

Moukoko CE, Elwali NMA, Saeed OK, Mohamed-Ali Q, Gaudart J, Dessei AJ, Chevillard C (2003). No Evidence for a Major Effect of Tumor Necrosis Factor Alpha Gene Polymorphisms in Peripotal Fibrosis Caused by Schistosoma mansoni Infection. *Infec. Immunity*. 17 (10): 5456-5460.

Rahoud S, Mergani A, Khamis AH, Saeed OK, Mohamed-Ali Q, Dessein AJ, Elwali NE (2010). Factors controlling the effect of praziquantel on liver fibrosis in Schistosoma mansoni infected patients. *FEMS Immunol. Med. Microbiol*.58 (1):106-112.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular cloning: A Laboratory Manual, 2nd ed. *Cold Spring Harbor Lab. Press, New York*, pp 3-105.

Secor WE, del Corral H, dos Reis MG, Ramos EA, Zimon AE, Matos EP (1996). Association of hepatosplenic schistosomiasis with HLA-DQB1*0201. *J. Infect. Dis*. 174: 1131-5.

Sleigh AC, Mott KE, Hoff R, Barreto ML, Mota EA (1985). Three-year prospective study of the evaluation of mansoni schistosomiasis in northeast Brazil. *Lancet* 2: 63-66.

Tamai K, Ishikawa H, Mauviel A & Uitto J (1995). Interferon gamma coordinately upregulates matrix metalloprotease (MMP)-1 and MMP-3 but not tissue inhibitor of metalloproteases (TIMP), expression in cultured keratinocytes. *J Invest Dermatol* 104: 384–390.

WHO (1993). The control of Schistosomiasi: Second report of the WHO expert committee. Genev, *WHO technical report series*, 830.

Wynn TA, Cheever AW, Williams ME, Hieny S, Caspar P, Kuhn R, Muller W, Sher A (1998). IL-10 regulates liver pathology in acute murine Schistosomiasis mansoni but is not required for immune down-modulation of chronic disease. *J Immunol* 160:4473–4480.

Xu JW, Gong J, Chang XM, Luo JY Dong L, Hao ZM, Jia A, Xu GP (2002). Estrogen reduces CCL4- induced liver fibrosis in rats. *World J.Gastroenterol*.8:(5)883-887.