



**Sudan University of Science & Technology  
College of Graduate Studies**

**Assessment of Praziquantel Effect and Some Genetic  
Factors on Prognosis of Periportal Fibrosis in Intestinal  
Schistosomiasis Patients in Gezira State**

تقويم عقار البرزاقونتل وبعض العوامل الوراثية التي تنبئ بتليف الوريد  
البابي في مرضى البلهارسيا المعوية في ولاية الجزيرة

**A Thesis Submitted in Fulfillment of the Requirements  
for the Degree of Doctor of Philosophy (PhD) in Medical  
Laboratory Science (Parasitology)**

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# *Dedication*

*To my  
Great Husband Moutz  
faithful friend Amria  
sister  
Brother  
Dad and Mam*

# Acknowledgements

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## Abstract

*Schistosoma mansoni* infection in Sudan causes serious health problems and economic consequences especially among schoolchildren, women of reproductive age and the working group of agriculture-associated people. Praziquantel has been introduced as the drug of choice for control of infection.

The aim of this study was to cast a light on the possible effect of praziquantel and some genetic factors on regression of the grade of Periportal fibrosis (PPF) 12 months after treatment of infected patients with *S.mansoni*.

The study enrolled 100 Sudanese nationals diagnosed as schistosomiasis patients in Um-Zukra with active *S.mansoni* infection complicated with different grades of PPF were enrolled in the study. All the participants underwent abdominal ultrasonography evaluating the grade of PPF and given praziquantel as treatment according to appropriate dose for each of them (40 mg/kg BW). They were further assessed by ultrasonography 12 months later.

The study found that, 46 (46%) grades of PPF regressed to the lower grades of fibrosis, 34 (34%) remained stable and 20 (20%) progressed to the higher grades of fibrosis. Age and gender were significantly associated with changes in grades of PPF. Regression of PPF among females was more frequent than in males patients.

Three polymorphisms (IFN- $\gamma$  rs2069705 (C/T), TGF-1+869 T/C and ILB-1-31T/C) that had been screened to assess their role in regression or progression of PPF after praziquantel therapy showed that there was no significant association between this polymorphism and disease prognosis (P.value :0.35). However, TGF- 1 +869 T/C- T/T homozygous and C/T heterozygous genotypes were more frequent in subjects with low grade of fibrosis (72.7%, P.value :0.029) (F I).

Similarly (ILB-1 31 T/C) TT homozygous and CT heterozygous genotypes were significantly more frequent in subjects with low grade of fibrosis (P.value :0.0001). In conclusion, while polymorphism may not contribute to PPF prognosis, yet it could be a predisposing factor.

## ملخص

مرض البلهارسيا المعوية في السودان من الأمراض الطفيلية الخطيرة و المنتشرة التي تهدد صحة الإنسان خصوصا الشباب واطفال المدارس والنساء في عمر الانجاب وعمال الزراعة مما ينعكس سلبا على اقتصاد البلاد في تلك المناطق الموبوءة من العالم. وحسب تقديرات منظمة ا لصحة العالمية فان حوالي مئاتي مليون شخص مصابون بالمرض ، عشرون مليون منهم يعانون من مضاعفات المرض الحادة .يستخدم عقار البرازوكونتيل لعلاج مرض البلهارسيا المعوية.

أجريت هذه الدراسة في إحدى قرى السودان الموبوءة بمرض البلهارسيا المعوية (قرية أم ذكرى بوسط السودان) بهدف دراسة تأثير عقار البرازوكونتيل وبعض العوامل الجينية التي تتحكم في عملية تليف الكبد وإمكانية تراجع التليف الي درجة أقل.

شملت الدراسة مائة مريضاً حددت درجات تليف الكبد لديهم بواسطة الموجات الصوتية قبل وبعد 12 شهراً من العلاج بواسطة عقار البرازوكونتيل. أشارت الدراسة إلى أن 46% من المرضى قد استجابوا للعلاج بدرجات متفاوتة وأن تليف الكبد لديهم قد تراجع الي درجة أقل بينما لم يتطور المرض في 34% من المرضى . أيضا هنالك 20% من المرضى تطور تليف الكبد لديهم إلى درجات أعلى.

أشارت الدراسة أيضا إن عوامل مثل عمر المريض والنوع (ذكر أو أنثى) ودرجة تليف الكبد وربما عوامل وراثية تتحكم في تراجع تليف الكبد الي الدرجات الأفضل.تم ايضا اختبار بعض الموسومات الجينية لمعرفة علاقتها بتطور او انحسار درجة التليف الكبدى مثل

(IFN- $\gamma$  rs2069705 (C/T), TGF-1+869 T/C and ILB-1-31T/C) وقد وجدنا ان ( TGF-1+869 T/C and ILB-1-31T/C ) اكثر انتشارا بين مرضى التليف الاقل درجة [F1] و (IFN- $\gamma$  rs2069705 (C/T) ليس لة علاقة بزيادة او تراجع درجات التليف بين مجموعة المصابين بمرض البلهارسيا المعوية با قرية أم ذكرى بوسط السودان

# **CHAPTER ONE**

## **INTRODUCTION**

## 1.1 Introduction

Schistosomes are trematodes that are typically digenetic but atypical to other trematodes in being dioecious (WHO ,1993). The main *Schistosoma* species in man are *S. mansoni*, *S. haematobium* and *S. japonicum*. The first two species are common in Sudan causing schistosomiasis; one of the most endemic diseases causing high morbidity and mortality (Tanner , 1989). The bodies of freshwater being natural or manmade such as in the irrigated agricultural schemes act as main reservoirs. Schistosomiasis due to *Schistosoma mansoni* is very common in Gezira scheme; the main irrigated agricultural scheme in Sudan

Despite the control efforts in a number of countries, still an estimated 200 million people are infected, of whom 120 million are symptomatic and 20 million have severe form of the disease. Due to its chronic nature, the disease affects labor capacity, and this has a major negative impact on socio-economic development of many endemic regions ((El-Wali, 2002 ).

Sudan is an agricultural country, and in all agricultural schemes, schistosomiasis is now believed to be endemic. During 1980 – 1990, a comprehensive control program was implemented in the Gezira area, central Sudan, that program is named as Blue Nile Health Project ( BNHP ). The program was able to reduce the prevalence of schistosomiasis to less than 10 %. At the moment, there is no proper active control program in all parts of the Sudan, and the disease is believed to be neglected. The prevalence of the schistosomiasis was increased to 70% in some areas. Treatment with Praziquantel tablets is

the only control measure available in the hospitals and health units.

According to hospital records, increasing number of patients suffering from most severe hepatic complications such as advance hepatic fibrosis which leads to portal hypertension, esophageal varices, massive haematamisis and death, was reported.

Heavy and chronic infections commonly result into portal hypertension with very serious fatal consequences such as the bleeding oesophageal varices( Homeida ,*et al* .,1991) The adult couple of *Schistosoma mansoni* travels from the portal vasculature in the liver against the blood stream to reside commonly in the venous of the mesenteric veins in the large intestine and may live for a decade or more. After mating each female (measuring about 1.1cm by 0.2mm) lays about 300 eggs per day ((Mohamed-Ali ,*et al* ., 1999). Some of the laid eggs are passed in stool, the other portion retained in the intestinal wall and the remaining eggs are gushed back to the liver. This last portion of the egg load constitutes the main pathogenic stage in schistosomiasis (Doehring ,*et al* .,1990). The eggs react immunologically with the immune system of the body leading to formation of granulomas around the eggs in the small portal venules of the liver ending up into peri-portal fibrosis (PPF) in the liver (Homeida ,*et al* .,1996). The peri-portal fibrosis obstructs with different grades the flow of the portal blood through the liver to the inferior vena cava leading to portal hypertension (Homeida ,*et al* .,1996). The peri-portal fibrosis comes out due to deposition of collagen (Wynn ,*et al* .,1998)round the portal tract giving three grades, grade I, II, and III (Wynn ,*et al* .,1998) It has been reported that successful treatment with praziquantel leads to regression of peri-portal fibrosis, However, only a small percentage of individuals infected in an area of endemicity actually develop a severe form of the disease. The severity of the disease is a

consequence of the severity of fibrosis( Homeida , *et al* .,1991) . It has been reported that the severity of fibrosis depends on the regulation of cytokines( Leptak & McKerrow ,1997). The T-cell-mediated host reaction to these eggs results in the formation of granulomas, which in turn produce the pathological consequences of hepatic fibrosis and portal hypertension. These granulomas consist of T cells, B cells, macrophages, fibroblasts, and a large number of eosinophils, and though largely mediated by Th2 cells, T helper 1- (Th1)-type responses can contribute to the tissue destruction ( Leptak & McKerrow , 1997).Early responses to the egg antigens are of both the Th1 type and Th2 type, with a subsequent shift to a long-lasting Th2 response. Recalling that a Th2 response is associated with the production of interleukin-10 (IL-10); IL-10 down-regulates interleukin-5 (IL-5) and interferon- $\gamma$ , significantly reducing liver damage associated with the granulomas ( Leptak & McKerrow, 1997). IL-10 also reduces the expression of the costimulatory molecules CD80 and CD86 on the local macrophages. Presentation of egg antigens in the absence of these CD28 costimulatory molecules leads to T-cell anergy, thus reducing the intensity of the inflammatory response generated against eggs antigens. Similarly, continuous activation of TH2 cells yields considerably high amounts of interleukin-4 (IL-4). IL-4 activates B cells to elicit a high level of immunoglobulin E (IgE) antibody during human infection ( Leptak & McKerrow , 1997). The orally administered praziquantel (PZQ) is the drug of choice for treatment of schistosomiasis and the granulomatous reaction that develops around the dead parasites causes complete disintegration of the parasite within two weeks and later on resolves the fibrotic lesions around the egg (Cheever. *et al* .,1994) In human *schistosomiasis* many reports had mentioned the antifibrogenic effect of IFN- $\gamma$  in hepatic fibrosis (Czaja, *et al* .,1989). Recent studies have

shown that human susceptibility to *S. mansoni* infection was controlled by two distinct genetic loci: *SM1* located in chromosome 5q31-q33 which controls the infection levels in Brazilian population (Dessein , *et al .*,1999)and *SM2* that is closely linked to interferon gamma receptor one (IFNGR1), the gene encoding the alpha chain of the IFN- $\gamma$  receptor, which controls the disease progression in Sudanese population. In a study done recently in Taweela population (Sudan), PPF has been shown to be associated with an increase in TNF- $\alpha$  production (El -Wali, *etal.*,2001 )and the progression to severe PPF in Schistosomiasis was found to be not associated with polymorphisms in the TNF- $\alpha$  gene (Henri S, *et al .*,2002). It has also been reported that hepatomegaly associated with or without splenomegaly in patients with *S. mansoni* infection is influenced by HLA (Dessein ,*et al .*,1999). *SM2* locus was found to be neither linked to *SM1* nor to the HLA locus . Base on the above finding, and since *SM2* locus was reported to control the progression of the disease (Homeida, *et al .*,1991) we suggest that the regression of PPF ( reversibility) is also under genetic control.

The aim of this study is to assess the Association pattern of change in the periportal fibrosis of *Schistosoma mansoni* infected Sudanese patient with PPF in Um zkra village in Al Managil, Central Sudan After PZQ treatment with fibrosis grade ,gender and some inherited genetic factor .

## **1.2 Rationale**

The shistosomiasis complication cause high mortality and morbidity rates in the endemic areas particularly in the central sudan . The polymorphisms in cytokine genes have allowed for the understanding of the genetic determinants of diseases. It was reported previously in a study done in Sudanese population of endemic area for *S. mansoni*, that two polymorphisms located in the third intron of the IFN- $\gamma$  gene are



associated with PPF. The IFN- $\gamma$  +2109 (A/G) polymorphism is associated with higher risk for developing PPF, whereas the IFN- $\gamma$  +3810 (G/A) polymorphism is associated with less risk for developing PPF. These polymorphisms result in changes in nuclear protein interactions with the intronic regions of the gene, suggesting that they may modify IFN- $\gamma$  mRNA expression (Chevillard, et al., 2003). In different study on the same population, Moukoko et al, (2003) found no evidence of association between four polymorphisms of TNF- $\alpha$  gene (TNF- $\alpha$  -376 G/A, -308 G/A, -238 G/A, and +488 G/A) and PPF. The polymorphisms studied of TNF-alpha (-308G/A), TGF-beta1 (codon 10C/T, codon 25C/G), IL-10 (-1082A/G; -819T/C; -592A/C), IL-6 (-174G/C), and IFN-gamma (+874T/A).were investigated in fibrogenesis shown with chronic pancreatitis (CP) and healthy individuals from in Brazil. The study conclude that The genotypes corresponding to the high TGF-beta1 producer phenotypes can be associated with the fibrogenesis shown with chronic pancreatitis CP"(Bendicho, etal.,2005).In this study we well investigate correlation of certain single nucleotide polymorphism (SNP), with PPF cause by *schistosoma mansoni* infection

### **1.3. Objectives**

#### **1.3.1. General Objective**

The aims of this study were:

To determine the correlation of certain factors that can effect the development of liver fibrosi in human intestinal Schistosomiasis after Praziquantel treatment

#### **1.3.2 Spesific Objectives**

- 1- To evaluate the effect of Praziquantel on the regression of liver fibrosis in an endemic population (UM Zukra village).
- 2- To identify the factors that may affect the regression of hepatic fibrosis (e.g. gender, age and grade of fibrosis).
- 3- To investigate the possible genetic polymorphisms in certain candidate genes such as interlukin-1 (IL-1) and TGF as profibrogenic and Gamma interferon (IFN- $\gamma$ ) as antifibrogenic and try to correlate these polymorphisms to the regression and progression phenotypes using different genetic and molecular tools. PCR followed by RFLP for these genes will be applied for differentiation.

# **CHAPTER TWO**

## **LITERATURE REVIEW**

# Introduction

Schistosomes are trematodes that are typically digenetic but atypical to other trematodes in being dioecious (WHO ,1993). The main Schistosoma species in man are *Schistosoma mansoni*, *Schistosoma haematobium* and *Schistosoma japonicum*. The first two species are common in Sudan causing schistosomiasis; one of the most endemic diseases causing high morbidity and mortality (Tanner M 1989). The bodies of freshwater being natural or manmade such as in the irrigated agricultural schemes act as main reservoirs. Schistosomiasis due to *S. mansoni* is very common in Gezira scheme; the main irrigated agricultural scheme in Sudan

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According to hospital records, increasing number of patients suffering from most severe hepatic complications such as advance hepatic fibrosis which leads to portal hypertension, esophageal varices, massive haematemesis and death, was reported.

Heavy and chronic infections commonly result into portal hypertension with very serious fatal consequences such as the bleeding oesophageal varices( Homeida . *et al* ;1991)The adult couple of *S. mansoni* travels from the portal vasculature in the liver against the blood stream to reside commonly in the mesenteric veins of the large intestine and may live for a decade or more. After mating each female (measuring about 1.1cm by 0.2mm) lays about 300 eggs per day (Mohamed *et al* ;1991). The majority of the laid eggs pass in stool, other portion retain in the intestinal wall and the remaining eggs gush back to the liver. This last portion of the egg load constitutes the main pathogenic stage in schistosomiasis (Doehring *et al* ;1990) The eggs react immunologically with the immune system of the body leading to formation of granulomas around the eggs in the small portal venules of the liver ending up into peri-portal fibrosis (PPF) in the liver(Homeida *et al* ;1996). The peri-portal fibrosis obstructs, with different grades the flow of the portal blood through the liver to the inferior vena cava leading to portal hypertension(Homeida *et al* ;1996) The peri-portal fibrosis comes out due to deposition of collagen (Wynn *et al* ;1995)round the portal tract giving three grades, grade I, II, and III (Wynn *et al* ;1995) It has been reported that successful treatment with praziquantel leads to regression of peri-portal fibrosis, However, only a small percentage of individuals infected in an area of endemicity actually develop a severe form of the disease. The severity of the disease is a consequence of the severity of fibrosis( Homeida . *et al* ;1991) · It has been reported that the severity of fibrosis depends on the regulation of cytokines( Leptak C L and

McKerrow J H 1997). The T-cell-mediated host reaction to these eggs results in the formation of granulomas, which in turn produce the pathological consequences of hepatic fibrosis and portal hypertension. These granulomas consist of T cells, B cells, macrophages, fibroblasts, and a large number of eosinophils, and though largely mediated by Th2 cells, T helper 1- (Th1)-type responses can contribute to the tissue destruction ( Leptak C L and McKerrow J H 1997). Early responses to the egg antigens are of both the Th1 type and Th2 type, with a subsequent shift to a long-lasting Th2 response. Recalling that a Th2 response is associated with the production of interleukin-10 (IL-10); IL-10 down-regulates interleukin-5 (IL-5) and interferon- $\gamma$ , significantly reducing liver damage associated with the granulomas ( Leptak C L & McKerrow J H 1997). IL-10 also reduces the expression of the costimulatory molecules CD80 and CD86 on the local macrophages. Presentation of egg antigens in the absence of these CD28 costimulatory molecules leads to T-cell anergy, thus reducing the intensity of the inflammatory response generated against eggs antigens. Similarly, continuous activation of Th2 cells yields considerably high amounts of interleukin-4 (IL-4). IL-4 activates B cells to elicit a high level of immunoglobulin E (IgE) antibody during human infection ( Leptak C L and McKerrow J H 1997).

The orally administered praziquantel (PZQ) is the drug of choice for treatment of schistosomiasis and the granulomatous reaction that develops around the dead parasites causing complete disintegration of the parasite within two weeks and later on resolves the fibrotic lesions around the egg (Cheever *et al* ;1994) In human schistosomiasis many reports had mentioned the antifibrogenic effect of IFN- $\gamma$  in hepatic fibrosis (Czaja *et al* ;1989. Studies have shown that human susceptibility to *S. mansoni* infection was controlled by two distinct

genetic loci: *SM1* located in chromosome 5q31-q33 which controls the infection levels in Brazilian population (Dessein *et al* ;1999) and *SM2* that is closely linked to IFN- $\gamma$  receptor one (IFNGR1), the gene encoding the alpha chain of the IFN- $\gamma$  receptor, which controls the disease progression in Sudanese population. In a study done in Taweela, PPF has been shown to be associated with an increase in TNF- $\alpha$  production (Elwali *et al*;2001) and the progression to severe PPF in schistosomiasis was found to be not associated with polymorphisms in the TNF- $\alpha$  gene (Henri S, *et al* 2002). It has also been reported that hepatomegaly associated with or without splenomegaly in patients with *S. mansoni* infection is influenced by HLA (Dessein *et al* ;1999). *SM2* locus was found to be neither linked to *SM1* nor to the HLA locus. Based on the above findings, and since *SM2* locus was reported to control the progression of the disease (Homeida *et al* ;1991) may be the regression of PPF (reversibility) is also under genetic control.

## **1.2 Rationale**

The schistosomiasis complications includes high mortality and morbidity rates in the endemic areas particularly in Central Sudan. The polymorphisms in cytokine genes have allowed understanding of the genetic determinants of the disease. It was reported previously in a study done in Sudanese population of endemic area for *S. mansoni*, (Dessein *et al* ;2003) that two polymorphisms located in the third intron of the IFN- $\gamma$  gene are associated with PPF. The IFN- $\gamma$  +2109 (A/G) polymorphism is associated with higher risk for developing PPF, whereas the IFN- $\gamma$  +3810 (G/A) polymorphism is associated with less risk for developing PPF. These polymorphisms result in changes in nuclear protein interactions with the intronic regions of the gene, suggesting that they may modify IFN- $\gamma$  mRNA expression (Chevallard *et*

al, 2003). In different study on the same population, Moukoko et al, (2003) found no evidence of association between four polymorphisms of TNF- $\alpha$  gene (TNF- $\alpha$  -376 G/A, -308 G/A, -238 G/A, and +488 G/A) and PPF. The polymorphisms studied of TNF-alpha (-308G/A), TGF-beta1 (codon 10C/T, codon 25C/G), IL-10 (-1082A/G; -819T/C; -592A/C), IL-6 (-174G/C), and IFN-gamma (+874T/A).



## **1.3 Objectives**

### **1.3.1 General Objective**

To assess the factors that can effect the development of liver fibrosis due to intestinal Schistosomiasis in human after Praziquantel treatment

### **1.3.2 Specific Objectives**

- 4- To evaluate the effect of Praziquantel on the regression of liver fibrosis in an endemic population in Um Zakra village .
- 5- To identify the factors that may affect the regression of hepatic fibrosis (e.g. gender ,age and grade of fibrosis).
- 6- To investigate the possible genetic polymorphisms in certain candidate genes such as TGF+14869 (T/C) and IL -1B-31(T/C) , Gamma interferon IFN rs2069705 (C/T)s

# LITRETURE REVIEW

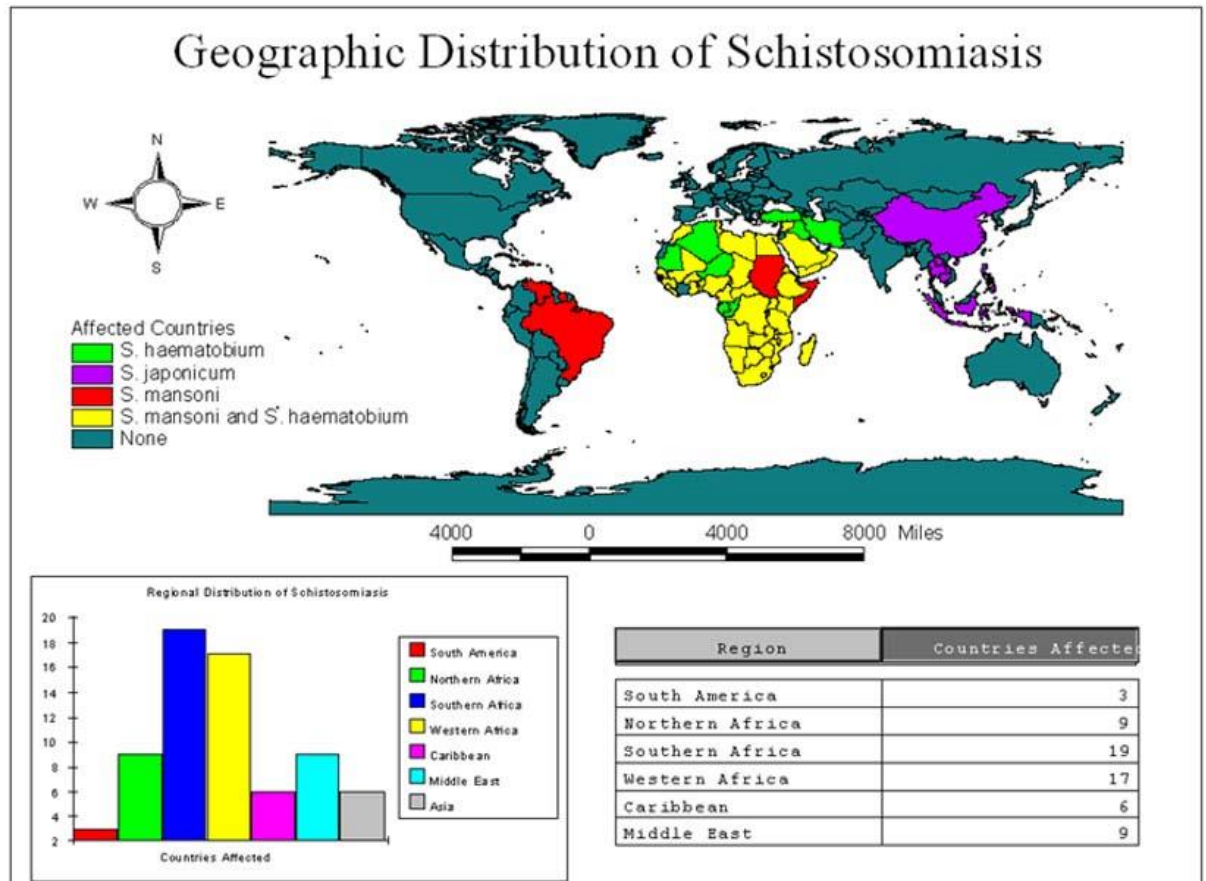
## 2.1Epidemiology and Etiology

Schistosomiasis is a chronic, parasitic disease caused by blood flukes (trematode worms) of the genus *Schistosoma*. At least 243 million people required treatment in 2011 that was repeated over a number of years ( WHO, 2011). Schistosomiasis transmission has been documented in 78 countries. However those requiring treatment targeted at most at-risk population groups live in 52 countries. Schistosomiasis is acquired by swimming, wading, or bathing in fresh water that is contaminated with the free-swimming stage of the parasite. Schistosomes multiply inside specific types of water-dwelling snails, from which they are released to swim free in the water. If they encounter a person's skin, they burrow in and move through the blood stream to the lungs, where they mature into adult flukes. The adults pass through the blood stream to their final home in small veins in the bladder or intestine, where they may remain for years. The adult flukes lay large numbers of eggs in the walls of the intestine or bladder. The eggs cause local tissue damage and inflammation, which results in ulcers, bleeding, and scar tissue formation. Some eggs pass into the stool or urine. If a urine or stool of infected people enters fresh water, the miracidim hatches , and the parasite enters snails to begin the cycle ( WHO, 2011). Trematodes of the genus *Schistosoma* reside in the bloodstream of their definitive vertebrate hosts, where they avoid immune destruction and survive for years or decades. Despite their continued presence at intravascular locations for periods of years, the adult parasites themselves provoke remarkably little tissue damage or inflammation. In contrast, the eggs produced by adult schistosomes can cause

considerable tissue damage and stimulate intense inflammatory and immune reactions. Indeed, the clinical classification of schistosomiasis as hepatic, intestinal or urinary depends on which organ or system is most severely affected by parasite eggs. Frequently over 100 µm long and possessing a tough proteinaceous shell, schistosome eggs trapped in host tissues such as the liver are not readily eliminated.

Consequently, like other focal stimuli of chronic inflammation, parasite eggs become the focus of granulomatous inflammatory reactions known as granulomas. Persistence of eggs and the granulomas that surround them results in fibroblast activation, synthesis of extracellular matrix proteins, formation of intractable fibrotic scars and disruption of tissue architecture, ultimately leading to organ dysfunction and the clinical manifestations of schistosomiasis (Abbas et al., 1991).

Different species of *Schistosoma* parasites were reported world wide, but only five species can infect man; *S. mansoni*, *S. japonicum*, *S. intercalatum*, *S. mekongi* and *S. haematobium*. The first four species cause intestinal schistosomiasis and the last one causes urinary schistosomiasis. *S. mansoni* is found throughout most of sub-Saharan Africa, Egypt and Sudan, parts of the Middle East, northeastern parts of South America including Brazil and the Caribbean (*S. haematobium* is also prevalent in most parts of Africa, and in parts of the Middle East. *S. japonicum* occurs in China, Malaysia, the Philippines, and, to a small extent, in Indonesia. *S. mekongi*, a closely related species to *S. japonicum*, is found in the Mekong River in Southeast Asia (Figure 1.1). Some investigators consider this schistosome a member of the *S. japonicum* complex (Hotez and Feng, 1997). *S. intercalatum*, a parasite of cattle in West Africa, also occasionally infects people in the Cameroon, Gabon, Equatorial Guinea, Central African Republic, Chad, and Zaire (WHO, 1989).



**Figure 2.1:**The geographical distribution of *Schistosomiasis*(  
www.CDC.com)

## 2.2 Transmission of the Disease

### 2.2.1 The Life Cycle of the Parasite

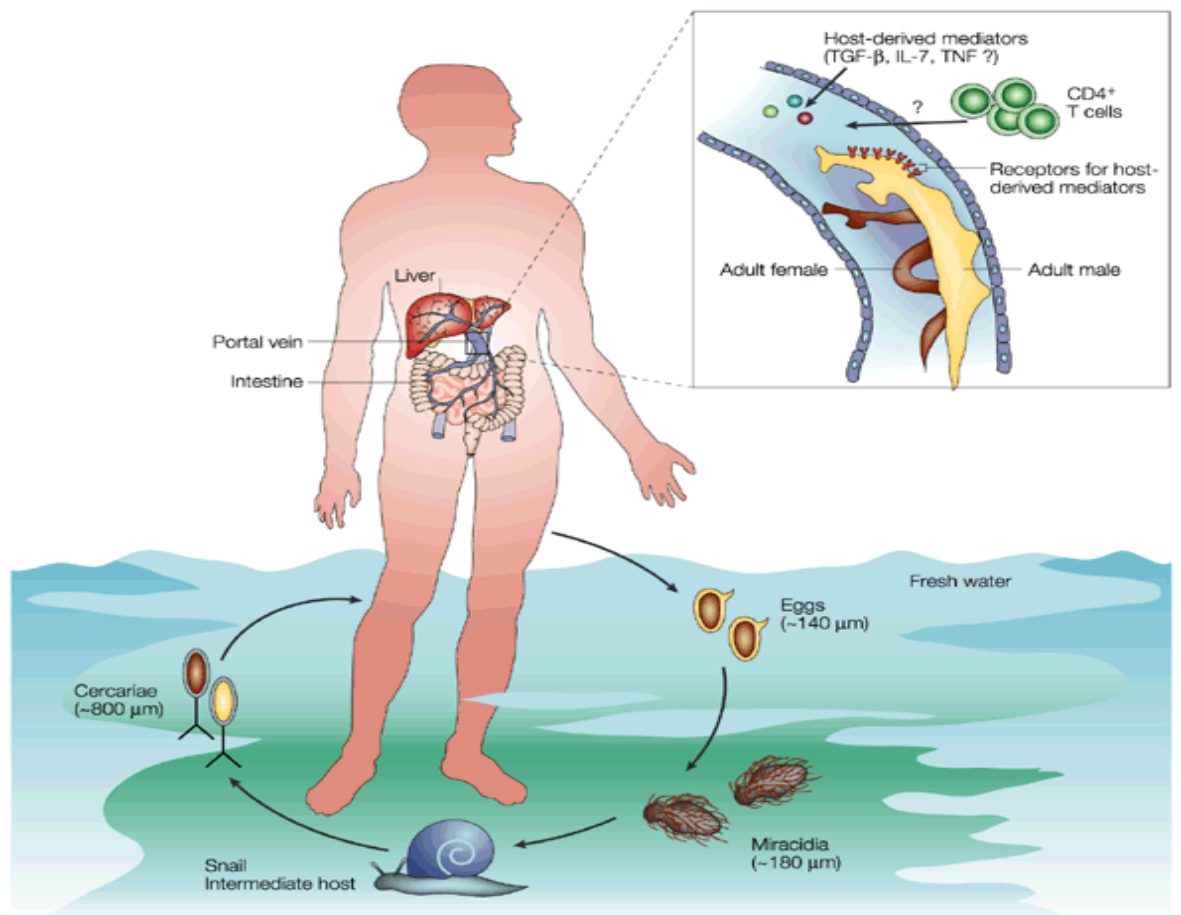
*Schistosoma* parasites (blood flukes) are digenic and dioecious trematodes. The adult worms remain in pairs during their life span (Figure 1.2), and live attached by their sucker disks to the endothelium of the abdominal veins of their vertebrate hosts. The parasite has a complicated life cycle alternating between aquatic snails (intermediate host), in which asexual reproduction takes place, and humans (definitive host), in whom sexual reproduction occurs (Figure 1.3) Human infection is initiated by cercarial skin penetration during exposure to water infested with cercariae through a variety of activities of daily living including wading, planting, fishing, swimming and washing. During this entry across the dermis, the cercarium loses its tail, and its head

transforms into a schistosomulum. The schistosomulum migrates up the venous system reaching the right heart, crosses the pulmonary capillary bed and, via the left heart, enters the systemic circulation. Few days later, each schistosomulum species demonstrates its preference for a specific venous location for mating and further development into adult worms. The hepatic forms (*S. mansoni* and *S. japonicum*) prefer the mesenteric vasculature, while the adult worms of *S. haematobium* prefer the draining venules of the urinary bladder. The small adult worms (male, 10 to 15, and female, 14 to 20, mm long) live in copula (Figure 1.2), and after four weeks, *S. mansoni* females produce, on average, 300 eggs per day, while *S. japonicum* and *S. mekongi* shed about 1500-3000 eggs daily. The life span of the worm is 5 to 10 years on the average, although some live as long as 37 years (Vermund et al., 1983). The eggs of *S. mansoni* are oval, and possess a lateral spine; those of *S. japonicum* and *S. mekongi* are globular and lack a spine; those of *S. haematobium* are oval, with a terminal spine (Figures 1.4).



**Figure 2.2:** Adult *S. mansoni* male and female in copula.

## Life cycle

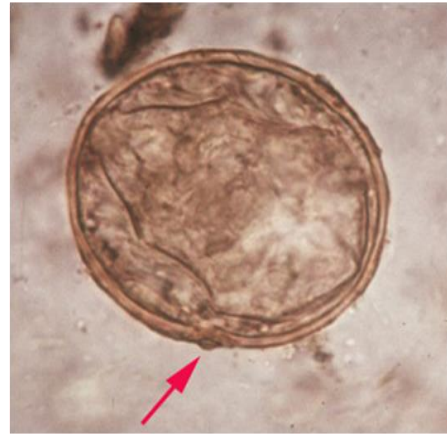


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**Figure 2.3:** The life cycle of *S. mansoni*. Transmission starts when water resources are contaminated with eggs which hatch to give miracidia that search for intermediate host (*Biomphalaria* snail) in which develop to cercaria (the infective stage).



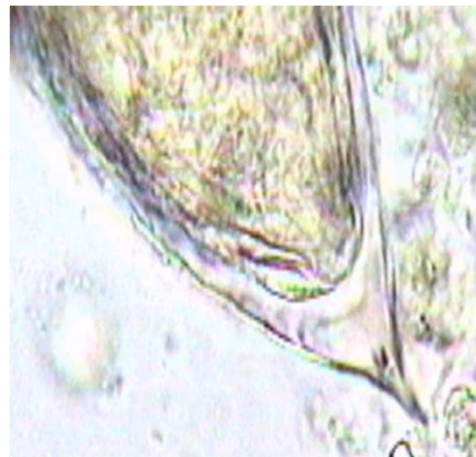
**A** ↑ (150 μm x 60 μm).



**B** ↑ (85 μm x 60 μm).



**C** ↑ (155 μm x 55 μm)



**C** ↑ (155 μm x 55 μm)

**Figure 2.4:** A: egg of *Schistosoma mansoni*, B: egg of *S. japonicum* and C: egg of *S. haematobium*

The worms feed on host erythrocytes and body solutes (Boros, 1989). Some of the eggs deposited by the females into the bloodstream pass through the venule walls, cross the intestinal mucosa, reach the lumen, and are evacuated with the fecal material. Eggs that arrive in the outside environment have to reach fresh water for the embryo (miracidium) to hatch and swim to find its intermediate snail host. For *S. mansoni*, the intermediate hosts are aquatic snails in the genus *Biomphalaria*. For *S. haematobium* and *S. intercalatum*, the aquatic intermediate host snails are in the genus *Bulinus* (Leiper, 1918). For *S. japonicum* and *S.*

*mekongi*, the amphibious intermediate host snails are in the genus *Oncomelania* (Miyairi and Suzuki, 1914).

Upon finding the right snail, the miracidium enters the soft, fleshy parts, facilitated by proteolytic enzymes. The miracidium invades the snail's lymph spaces, and then the hepatopancreas. A series of remarkable transformations then start, beginning with production of the sporocyst. This stage gives rise to daughter sporocysts, which, in turn, produce cercaria, the infectious stage for humans. During each stage of development, there is an increase in the number of individuals. A single miracidium of *S. mansoni* produces about 4,000 cercariae. This step of the cycle takes four weeks for *S. mansoni*. All offspring of one egg will be of one sex. The cycle is repeated by cercarial skin penetration (Figure 1.3).

### **2.2.2 The Intermediate Hosts of the Parasite in Sudan**

Schistosomiasis is endemic in many parts of the Sudan (Amin and Sati, 1973). Several malacological surveys were conducted in many parts of the Sudan. For *S. mansoni*, the intermediate host of genus *Biomphalaria* *alexandrina* was reported in the Gezira area (Greany, 1952a), *B. sudanica* in Southern Sudan (Malek, 1958), *B. pfeifferi* in Sennar reservoir (Williams and Hunter, 1968) and *Bio. ugandae* in Kosti (El-hussien, 1989). For *S. haematobium*, Greany, (1952a) reported *Bulinus truncatus*, *Bul. forskalii* and *B. africanus* in the Gezira area, Malek, (1958) reported *B. ugandae* and *B. globosus* in Southern Sudan.

## **2.3 Diagnosis of the Disease**

Diagnosis is important in all aspects of schistosomiasis. Decisions on individual and community treatment, estimations on prognosis and assessment of morbidity, evaluation of chemotherapy and control measures, and all these aspects build on the results from diagnostic tests.



### **2.3.1 Microscopical Diagnosis and Eosinophilia**

Microscopic identification of eggs in stool or urine is the most practical method for diagnosis. Stool examination should be performed when infection with *S. mansoni* or *S. japonicum* is suspected, and urine examination should be performed if *S. haematobium* is suspected. This method is time consuming and it has to be done by skilled personnel. Stool examination can be performed by simple saline smear (wet preparation) in heavy infection in which mixing (1 to 2 mg) of faecal material with normal saline and then examined under microscope using 10 or 40. Since eggs may be passed in small amounts, their detection will be enhanced by repeated examinations and/or concentration procedures. Using of quantitative Kato Katz method (Katz *et al*, 1972) is recommended because intensity of infection is associated with morbidity. Egg-hatching technique for detecting viable ova can be performed for treatment assessment. Eosinophilia was used as indicative method to diagnose active schistosomiasis infection (Wouter *et al*, 2005).

### **2.3.2 Serological Diagnosis**

#### **2.3.2.1 Antibody detection**

Antibody detection approach using the enzyme linked immunosorbent assay (ELISA), can be useful to indicate schistosome infection in patients who have traveled in schistosomiasis endemic areas and in whom eggs cannot be demonstrated in fecal or urine specimens. The sensitivity and specificity of the test is varied and are dependent on both the type of antigen preparations used (crude, purified, adult worm, egg or cercarial antigen) and the test procedures (Tsang and Wilkins, 1997).

#### **2.3.2.2 Antigen detection**

Schistosome worms and eggs excrete several antigens in the body that can be traced in urine or serum of infected individuals, using ELISA technique. Recently, a lateral flow based reagent strip was developed for diagnosis of schistosomiasis. The test is rapid (15 – 30 min.), sensitive and specific (Tsang and Wilkins,

1997; Wouter *et al*, 2005).

### **2.3.3 Molecular Diagnosis Using (PCR)**

Polymerase chain reaction (PCR) technique was used to diagnose human schistosomiasis by amplifying the parasite DNA in human faecal and serum samples. PCR assay might be a valuable alternative technique for diagnosing *Schistosoma* infections (Steiner *et al*, 1995; Pontes *et al*, 2002; Robello *et al*, 2002; Pontes *et al*, 2003).

### **2.3.4 Diagnosis by Medical Imaging**

#### **2.3.4.1 Ultrasonographical Diagnosis**

Many studies have shown that abdominal ultrasonography can be reliably used to diagnose hepatosplenic schistosomiasis and assess its severity (Babello *et al*, 1994; Abdel-Wahab *et al*, 1992). Different protocols have been developed for ultrasonographic examination of schistosomiasis. For *S. mansoni*, there are two methods in use for assessing the peribortal fibrosis. One is a descriptive method, which takes into account the liver texture as a whole (Homeida *et al*, 1988; Doehring *et al*, 1989). The other is a quantitative method, involving the measurement of the thickness of the walls of branches of the portal vein (Abdel-Wahab *et al*, 1992). WHO recommends both methods in a workshop on ultrasound in schistosomiasis held in Niamey, Niger (WHO, 1996). Diagnosis by ultrasound reveals characteristic periportal fibrosis and sometimes distention of portal and splenic veins exceeding 12 or 10 mm width (El-Zayadi, 2004; Mohamed-Ali *et al*, 1999; Dittrich *et al*, 1983; Abdel-Wahab, 1989).

#### **2.3.4.2 Magnetic Resonance Imaging (MRI)**

Magnetic resonance imaging is the newest medical imaging technology used to diagnose diseased tissues in early stage of the disease (Kevles, 1997; Olds and Dasarath, 2000).

### **2.3.5 Diagnosis by Endoscopy**

Bleeding from esophagogastric varices is a potentially deadly complication in patients with hepatosplenic schistosomiasis. The esophageal varices can be diagnosed by upper gastrointestinal endoscopy (Bhargava *et al*, 1989; Sakai *et al*, 1990; Olds and Dasarath, 2000; Martins *et al*, 2000).

### **2.3.6 Biopsical Diagnosis**

In tissue biopsy, eggs can potentially be demonstrated on liver or rectal specimens and are highly specific, but these procedures are now rarely performed. Liver biopsy shows Symmer's or pipe-stem fibrosis (John *et al*, 1996; El-Zayadi, 2004).

## **2.4 Treatment and Control of Schistosomiasis**

### **2.4.1 Treatment**

The main objectives of treatment with antischistosomal drugs are: to control the infection in the individual level, and to reduce the transmission of the disease in the community level (Davis, 1993). Prior to the 1960s, antischistosomal drugs were highly toxic. Antimony was used as an atibilharzial (antischistosomiasis) agent in 1918 in Egypt (McDonagh, 1918; Christopherson, 1918). The drug was used as intravenous injections. In the 1960s, the first oral drug became available is niridazole. Other agents include hycanthon, oxamniquine, and metrifonate, were used as antischistosomal drugs. Metrifonate is used for treatment of *S. haematobium* infections only, while oxamniquine is used for treatment of *S. mansoni* infections only (Davis, 1993). Praziquantel was introduced in 1972 and remains till now, the main effective and cheapest treatment for all species of schistosomiasis (Olds and Dasarath, 2000). PZQ treatment 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 (Homeida, et al, 1991 ). So collectively, PZQ prevent the formation of extra fibrous tissue. It is not known whether PZQ have an effect on existing fibrosis (Fibrolysis), but it is possible to activate the metalloproteinase enzyme which degrade the fibrosis tissue

#### **2.4.2 Control**

Transmission of schistosomiasis requires contact with water infested with cercaria. To stop or reduce the transmission of the disease, certain measures should be applied: first, reducing contact with infested water, and this will be achieved by snail control (chemical molluscicides and cleaning water canals from the vegetations) and provision of adequate and safe water supply. Second, reduce or stop contamination of water with schistosome eggs, and this will be achieved by treatment and health education (Webbe and Jordan, 1993).

### **2.5 The Pathophysiology of *S. mansoni* Infection**

The pathophysiology of *S. mansoni* infection can be divided in three stages:

#### **2.5.1 The Immediate Manifestations of the Disease (Dermatitis)**

Schistosome infection is initiated after cercariae penetrate through the skin and transform into schistosomula. Penetration and migration of cercariae are facilitated by proteolytic enzymes secreted from cephalic glands capable of digesting epidermal keratin (Fukuyama *et. al*, 1983). At the site of penetration by the cercariae, skin reactions eg.itching may develop within a few hours after infection. However, a rash may appear up to one week later. The dermatitis is similar to, but less severe than, swimmers' itch, which develops in sensitized persons when they are

reinfected by species of schistosomes that do not infect humans, usually the types that infect birds (Warren, 1973).

### **2.5.2 Acute Schistosomiasis (Katayama fever)**

Acute schistosomiasis is a clinical syndrome often seen in nonimmune individuals (tourists, immigrants, or indigenous population) who have been exposed in an endemic area to a primary infection by cercariae (Clarke *et al*, 1970; Zuidema, 1981). The syndrome is sometimes called Katayama fever, described in Katayama Valley of Japan, in persons who acquired a massive infection with *S. japonicum* worms. The acute intestinal symptoms due to *S. mansoni* infection appear when adult worm pairs begin releasing eggs in the tissues, and include diarrhea which may start suddenly 40 – 55 days after the primary infection and may last for 6 – 12 months. Patients may experience fever, headache, myalgia, abdominal pain, or cough as larvae migrate through tissues to develop in the bloodstream. Disease development and patient symptoms are caused by the host immune response to the eggs deposited into tissues rather than to the adult worms. Hypersensitivity to initial egg deposition can result in urticaria, generalised pruritus, rash, or facial oedema. Eosinophilia is common at this time. (El –Wali, 2002). Acute disease manifestations rarely occur among reinfected individuals living in an endemic area, presumably because of their pre- and postnatal exposures to schistosome antigens via transplacental transfer (Carlier *et al*, 1980), lactation (Grimaud *et al*, 1987), and repeated infections at an early age.

### **2.5.3 Chronic Schistosomiasis**

The classical manifestation of schistosome infection occurs as a consequence of many years of progressive injury resulting from chronic egg deposition in the tissues and the injury has an immunopathologic basis (Boros, 1989). In *S. mansoni* infection, eggs that do not reach the intestinal lumen are swept into the portal circulation and are trapped in

the intestinal wall causing the chronic intestinal form of the disease, during which patients complain of abdominal pain. In this stage, a case of chronic catarrhal with swollen granular hemorrhagic mucous membrane and numerous eggs in the mucosa and submucosa of the rectum was observed. Chronic hepatosplenomegaly in schistosomiasis is encountered more frequently in patients resident in areas of schistosomiasis endemicity showing high transmission rates of the disease, and the level of its occurrence has been associated with the intensity of infection (Arap Siongok *et al*, 1976; Gryseels, 1991). Eggs trapped in liver parenchyma, are surrounded by the granulomatous inflammatory response of the host, which evoked by the antigens that secreted by the eggs leading to the hepatosplenic form of the disease. Hepatomegaly reflects the presence of granulomatous inflammation and occurs early in the evolution of chronic disease. The intensity and duration of infection determine the amount of antigen released and the severity of chronic manifestation of the disease (Boros and Warren, 1970). Chronic infection presents a range of possible symptoms, from anaemia and malnutrition to obstructive uropathy or portal hypertension. Iron loss and chronic inflammation contribute to anaemia and malnutrition. Pathology caused by immune-mediated inflammatory granulomas formed around parasite eggs trapped in host tissues leads to fibrosis, obstruction, and dysfunction of the affected organ. Rarely, ectopic eggs in the CNS can result in cerebral masses or transverse myelitis with flaccid paralysis.

The granulomas destroy the ova but sometimes accompanied by excessive fibrous tissue

deposition which concentrates in the periportal spaces leading to the development of periportal fibrosis (Allen *et al*, 2002).

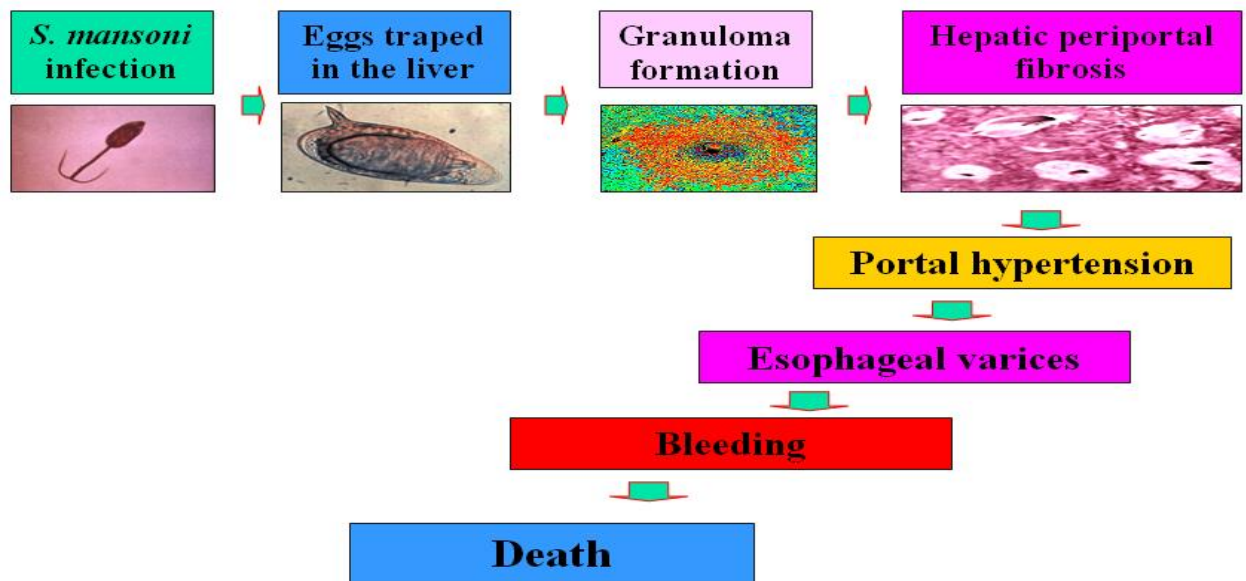
The most prominent feature of liver pathology in schistosomiasis is represented by a process of portal fibrosis that extends from the smallest to the largest portal spaces. It forms a typical gross finding that has sometimes been referred to as 'pipestem' fibrosis (Allen et al, 2002).

Periportal collagen deposits lead to the progressive obstruction of blood flow, portal hypertension, and ultimately varices, variceal bleeding, splenomegaly, and hypersplenism (Figure 1.6). Hepatosplenic schistosomiasis (HSS) is the best-known form of chronic disease and usually results from chronic heavy *S.mansoni* infection (Lambertucci, 1993; El-Zayadi, 2004). This condition occurs in 4 to 8 percent of patients who have chronic infection (WHO, 1998). Periportal granulomas have been found in many types of tissue, including the skin, lung, brain, adrenal glands, and skeletal muscle (King, 2001).

## **2.6 Granuloma Formation and Modulation**

Granuloma is a focal chronic inflammatory reaction. Hepatic schistosomiasis results from the host's granulomatous cell-mediated immune response to the soluble egg antigen of *S.mansoni*, which progresses to irreversible fibrosis and, consequently, severe portal hypertension (Vennervald et al., 2005). Eggs remain viable in the liver for about 3 weeks. Primarily, the eggs cause a moderate type 1 helper (Th1) response to egg antigens. However, this usually evolves to a dominant Th2 immune response to egg-derived antigens with later recruitment of eosinophils, granuloma formation and fibrogenesis of the liver.

Although granuloma formation is beneficial for the host because it blocks the hepatotoxic effects of antigen released from parasite eggs, this process may lead to fibrosis with excessive accumulation of collagen and extracellular matrix proteins in the periportal space.



**Fig 2.5:** The Possible Consequences of *S. mansoni* infection

When the volume of granulomas decreases around recently laid eggs containing viable mature miracidia, the phenomenon is referred to as granuloma modulation. Little is known of granuloma modulation in humans, but animal models reveal a complex interplay of regulatory cytokines, tumor necrosis factor, various immune effector cells, and fibroblasts, all subject to genetic influence. The balance of cellular and humoral influences on modulation may vary with the chronicity of the infection and with humoral activity dominating in the later stages of disease (Cheever, 1997). eosinophils are dominant in the early stage of the infection, but are lower during the acute and chronic stages of the disease (Pacheco and Lenzi, 1997).

In human schistosomiasis, (Coelho, 1955) granuloma divided in to three stages: first, characterized by focal histolytic and cellular exudation; second, characterized by productive or encysted histiocytic reaction, and third, is repair or healing stage which characterized by fibrous substitution.



In murine model, (Henrique *et al*, 1998) divided the granuloma in to two stages: first, the pre-granulomatous stage, which characterized by lytic proses to prepare the space through destruction of the parenchyma for the establishment of the second phase of the granuloma. The second stage, is the granulomatous phase which characterized by cellular adhesion and sorting out. Various type of cells exhibit different degrees of adhesion. Cells arrange themselves into specific patterns, including the sorting out of different types of cells from each other. The effects of aggregation and sorting create different zones in the granuloma structure. While the combination of different types of cells form the internal or peri-ovular layer, which enveloped by the paracentral and external layers (Henrique *et al*, 1998).

### **2.6.1 Cellular Components of the Granuloma**

The cellular components of granuloma around the viable egg (containing viable meracidium) include eosinophils, macrophages, lymphocytes, neutrophils, mast cells, and fibroblasts (Asahi *et al*, 1999). In addition to the above cellular components of the granuloma, Henrique *et al*, (1998) reported endothelial cells, monocytes, necrotic hepatocytes and giant cells. Studies in experimental animals show differences in cellular composition of granuloma according to tissue type. Hepatic granuloma contain the largest number of T and B lymphocytes, eosinophils, and mast cells, whereas ileal granulomas consist mainly of macrophages, and there are different patterns of distribution of T and B lymphocytes within granulomas in different tissues (Weinstock *et al*, 1983). The process of granuloma formation is dependent on CD4+ T helper lymphocytes (Wynn and Cheever, 1995) and results in a shift of the immunologic balance from a Th1 to a Th2 cell type response (Pearce *et al*, 1991; Grzych *et al*, 1991).

### **2.6.2 Role of Adhesion and Chemokine Molecules in Granuloma Formation**

The intercellular adhesion molecule-1 (ICAM-1), or CD54, is a cell-surface protein with five immunoglobulin-like domains that is expressed constitutively at low levels on vascular endothelial cells, lymphocytes, and monocytes. ICAM-1 participates in the adherence of inflammatory cells to the endothelium before diapedesis occurs .

The stimulation of a variety of cells, such as endothelial, mesangial, and liver epithelial cells, with inflammatory cytokines [interleukin (IL)-1, TNF- $\alpha$ , and IFN- $\gamma$ ] increases expression of ICAM-1

However, the exact role of ICAM-1 in the recruitment of leukocytes to the primary site of infections with the egg of *S.mansoni*, in the formation of granulomas, (Lukacs and Boros 1993).

intercellular adhesion molecule-1 (ICAM-1) and E-selectin are secreted by activated endothelial cells, and were reported in high levels in patients with hepatosplenic disease (Pigott *et al*, 1992; Secor *et al*, 1994).

In experimental animals, ICAM-1, lymphocyte function-associated antigen-1 (LFA-1), and very late antigen 4 (VLA-4) adhesion molecules were found to participate in initiation and maintenance of hepatic granuloma (Jacobs *et al*, 1997). The recruitment and accumulation of inflammatory cells around eggs in the granuloma are controlled by different chemokines. Macrophage inflammatory protein 1 $\alpha$  (MIP-1 $\alpha$ ) was identified as chemokine in macrophages in primary pulmonary granuloma in mice, and when this chemokine is blocked with antibody, the pulmonary granuloma was decreased. The production of monocyte chemoattractant protein-1 (MCP-1) by fibroblasts in granuloma was found to be correlates with the size of granuloma at different stages of infection (Lukacs and Boros 1993). Other chemokine, Regulated upon Activation by Normal T cell Expressed and Secreted (RANTES), which produced in granuloma, is involved in recruitment and activation of

eosinophils (Lukacs *et al*, 1994). In human schistosomiasis, RANTES was found to be associated with increased risk of fibrosis (Mark *et al*, 2004; Booth *et al*, 2004a).

### **2.6.3 Role of Cytokines in Granuloma Formation**

T cell-mediated granulomatous inflammation and irreversible fibrosis. In the murine model granulomatous inflammation is induced by CD4+ T helper. Mediator production was examined in splenocyte as well as granuloma cell cultures of infected or egg granuloma-bearing mice. IL-1 mRNA expression and IL-1 production were detectable within the first 4 days of granuloma growth. After 4-6 days TNF-alpha mRNA message appeared and cytokine production was observed. With the aging of the granuloma, production of both cytokines diminished. Thus, these cytokines are considered to be the primary recruiters of cellular aggregation in granuloma growth. The role of TNF-alpha in granuloma formation was also confirmed in infected mice. Whereas treatment of animals with anti-TNF-alpha antiserum diminished hepatic granuloma size, repeated injection of murine TNF-alpha into chronically-infected mice enhanced the downmodulated granuloma response (Minasian and Nicola, 1992; Casciari *et al*, 1996). IFN-gamma when administered in large doses diminished granulomatous inflammation, plays a regulatory role in the maintenance of the granulomatous response (Abram. B.S.2004)

#### **2.6.3.1 Role of Th2 Cytokines in Granuloma Formation**

Th2 is a subset of T-helper cells, which promote B-cell mediated humoral responses (Bidwell *et al*, 1999). Cytokines produced by Th2 cells include interleukin-4 (IL-4), IL-5, IL-6, IL-10, IL-13, and tumour necrosis factor alpha (TNF- $\alpha$ ). The roles of Th2 cytokines in granuloma formation and in fibrosis have been evaluated in experimental models of

schistosomiasis by many authors. IL-4 was shown to be strongly proinflammatory and stimulates the production of collagen and extracellular matrix proteins (ECMP) by stimulating the differentiation of stellate cells into myofibroblasts (Chesue *et al*, 1994; Cheever *et al*, 1994; Tiggelman *et al*, 1995). In murine schistosomiasis, IL-5 and IL-4 were reported to control the level of serum IgE and the peripheral and tissue eosinophilia (Grzych *et al*, 1991; Fallon *et al*, 2000). IL-10 regulates the excessive Th1 and Th2 polarization of the granulomatous response, and stimulates protection against fibrosis by increasing IFN- $\gamma$  production when administered with egg antigens (Wynn *et al*, 1995; 1997; Hoffmann *et al*, 2000).

Biochemical and molecular studies have shown that IL-13 is the major stimulus for the development of egg-induced liver fibrosis (Chiaramonte *et al*, 1999). In experimental animals, when the activity of IL-13 is blocked (IL-13<sup>-/-</sup> animals), the mortality is delayed indicating the major contribution of IL-13 to the progression of the chronic murine schistosomiasis (Fallon *et al*, 2000). In vitro studies demonstrated the ability of IL-13 to stimulate collagen deposition by fibroblasts (Chiaramonte *et al*, 1999). In human schistosomiasis, IL-13 has been reported to exhibit chemotactic activity for human eosinophils and may play a role in eosinophil survival by stimulating the production of granulocyte-macrophage colony stimulating factor (GM-CSF) (Mentink-Kane and Wynn, 2004).

Tumor necrosis factor alpha (TNF- $\alpha$ ) has been reported to modulate the granulomatous reaction induced by schistosome eggs (Amiri *et al*, 1992; Joseph and Boros, 1993). Several reports have suggested that TNF- $\alpha$  may increase hepatic fibrosis and severe disease in *S. mansoni* infection (Adewusi *et al*, 1996; Leptak *et al*, 1997). A high level of TNF- $\alpha$  was reported to be associated with a high risk of PPF (Henri *et al*, 2002). Th2

cytokines constitute the pro-inflammatory cytokine profile and have pro-fibrotic effect (Abbas *et al*, 1991; Mosmann, 1991; Hesse *et al*, 2000). In experimental models, the production of Th-2 cytokines by spleen cells and hepatic granuloma cells was predominant compared with Th1 cytokines, and there was cross- regulation of cytokine production: as Th2 cytokine production increased, Th1 cytokine production decreased (Grzych *et al*, 1991; Sher *et al*, 1991; Mosmann, 1991).

### **2.6.3.2 Role of Th1 Cytokines in Granuloma Formation**

Th1 is a subset of T-helper cells, which promote Cell-mediated effector responses (Bidwell *et al*, 1999). Cytokines produced by Th1 cells include IL-2, IL-12, and IFN- $\gamma$ . Th1 cytokines constitute the anti-inflammatory cytokine profile and have anti-fibrotic effect (Hesse *et al*, 2000; Wynn *et al*, 2004). IL-2 is involved in the pulmonary granuloma formation as growth factor for the Th2 response (Wynn *et al*, 1993). In experimental model, administration of IL-12 was reported to suppress the Th2 response by reducing IL-4, IL-5, IL-10 and increasing IFN- $\gamma$  production (Oswald *et al*, 1994). Inhibition of granuloma formation and reduction of tissue fibrosis were observed in C57BL/6 mice sensitised with both, eggs and IL-12 (Wynn *et al*, 1995a). Many authors reported IFN- $\gamma$  as a strong antifibrogenic cytokine. It inhibits the production of extracellular matrix proteins (ECMP) by stellate cells and increases the collagenase activity of the liver by stimulating metalloprotease (MP) synthesis and by inhibiting the synthesis of tissue inhibitors of MP (TIMP) (Doncan *et al*, 1985; Tamai *et al*, 1995).

### **2.6.3.3 Role of Th3 Cytokines in Granuloma Formation**

Th3 is a subset of T-helper cells which has been defined recently in human and characterized by transforming growth factor beta (TGF- $\beta$ ) production (Bidwell *et al*, 1999; Abbas *et al*, 1991; Mosmann, 1991).

TGF- $\beta$  is produced in several isoforms by Ito (stellate) cells and by other nonparenchymal cells during fibrogenesis in the liver (Branton and Kopp, 1999). TGF- $\beta$  was reported as fibrogenic cytokine, and its association with hepatic fibrosis based on a variety of evidence. Treatment of cultured hepatic cell with TGF- $\beta$ 1 increased the level of type 1 procollagen mRNA, and an increase in TGF- $\beta$ 1 gene expression preceded the increase in collagen synthesis (Czaja *et al*, 1989). It stimulates the differentiation of stellate cells into myofibroblasts and it exerts effect opposite to IFN- $\gamma$  (Roberts *et al*, 1986; Tiggelman *et al*, 1995).

#### **2.6.4 Fibrosis, Fibrogenesis and Fibrolysis**

Fibrosis is an accumulation of fibrous tissue resulting from an imbalance between several types of liver cells. Fibrosis develops in granulomas during the chronic phase of granulomatous inflammation due to schistosomiasis. Fibrogenesis is a dynamic process regulated by cytokines produced by macrophages, lymphocytes, and fibrocytes (Chesney *et al*, 1998; Wyler, 1996).

##### **2.6.4.1 The Extracellular Matrix (ECM) Formation**

###### **(Fibrogenesis)**

The ECM is a relatively stable structural material that lies under epithelial and surrounds connective tissue cells. The biological functions of the ECM are related to determination of the cytoskeleton, cell shape, cell migration, control of cell growth and cell differentiation (El-Wali, 2000). The major cells involved in the liver fibrosis are Stellate cells. Under normal conditions, stellate cells (star shape cells, , which are localized in the space of perisinusoidal spac, are reservoirs of fat and vitamin A in the liver. They also contain filaments that can contract and regulate blood flow through the liver (Gressner and bachem, 1995;

Gressner, 1991). Under activation, the hepatic stellate cells (HSC) proliferate, lose their vitamin A and undergo a major phenotypical transformation to smooth muscle  $\alpha$ -actin positive myofibroblasts (activated HSC), which produce a wide variety of collagenous (collagen I, III, IV, V and VI) and non-collagenous ECM proteins which include proteoglycan, fibronectin, laminin, and hyaluronan (Gressner and Harman, 1988; Maher *et al*, 1988; Gressner, 1991; Gressner and bachem, 1995; Ramm *et al*, 1995). The activation and transformation of HSCs are mediated by cytokines such as platelet derived growth factor (PDGF), transforming growth factor beta (TGF- $\beta$ ) and IL-4 as main fibrogenic mediators (Peterson and Isbrucker, 1992; Gressner, 1991).

#### **2.6.4.2 Degradation of the Extracellular Matrix (Fibrolisis)**

Hepatic fibrosis is a pathological process with the overlapped extracellular matrix protein (ECMP). Fibrosis develops either when there is increase in ECM synthesis, or when there is inhibition of ECM degradation . The degradation of ECM is mainly regulated by matrix metalloproteinases (MMPs). Synthesis of MMPs in the liver is stimulated by IFN- $\gamma$  leading to increase in ECM degradation. The activity of MMPs is inhibited by tissue inhibitors of MMPs (TIMPs), leading to increase in ECM synthesis and finally to fibrosis. The activity of TIMPs is inhibited by IFN- $\gamma$  as anti-fibrogenic cytokine (Doncan *et al*, 1985; Tamai *et al*, 1995).

#### **2.7 Periportal Fibrosis (PPF)**

Periportal fibrosis of the liver is a serious consequence of *S. mansoni* infection that involves remodelling of the ECM and excessive deposition of collagen along the branches of the portal tract (Booth *et al*, 2004). It is formed due to the granulomatous reactions around the schistosome eggs in the liver, and it represents one of the main mechanisms of tissue repair

(Grimaud and Lortat-Jacob, 1994). Egg granulomas produce a variety of factors (fibroblast-stimulating factor, IL-4 and TGF- $\beta$ ) that induce production of ECM by myofibroblasts (Wyler *et al*, 1978; Gressner, 1991). Fibrosis occurs when there is an imbalance between fibrogenesis and fibrolysis. Increase deposition of ECM in the liver leads to obstruction of portal vessels, and this result in portal hypertension that leads to collateral circulation and esophageal varices, which often their bleeding leads to death (Lambertucci, 1993; Brenner *et al*, 2000).

The biochemical composition of the fibrous tissue in the liver during the course of *S. mansoni* infection is changed according to the stage of the disease. In the acute phase, the dominant components of the fibrous tissue are collagen type I and glycosaminoglycan. In the chronic phase, collagen type III and its collagenases are dominant while the synthesis of type I collagen and glycosaminoglycan decreases (El-Meneza *et al*, 1989).

#### 2.8 Genetic Susceptibility to Schistosomiasis

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, 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). Various studies have attempted to identify the factors that cause disease to develop in only a fraction of the population exposed to parasites. Linkage analysis, a molecular genetic tool, in which highly polymorphic markers distributed throughout the genome are used to identify chromosomal regions that segregate with disease susceptibility. Linkage analysis is the preferred method for identifying genes that exert a major effect on disease susceptibility (Marquet *et al*, 1996; 1999; Kwiatkowski, 2000). It has been reported previously that, the infection levels and the prevalence of *S. mansoni* infection in endemic populations depend mainly on the resistance/susceptibility of the human host and the infection levels have been shown to be controlled by a locus (*SMI*) that maps to chromosome 5q31-q33 (Marquet *et al*, 1996; 1999; Dessein *et al*, 1999). The *SMI* locus



contains a number of genes that encode many cytokines that play an important role in the regulation of the immune response against helminth parasites (Dessein *et al*, 2001). In a study done in Sudan, in area endemic for schistosomiasis, the levels of the infection were found to be one of several factors important in disease development, suggesting that the progression of the disease and the level of the infection were not necessarily regulated by the same factors (Mohamed-Ali *et al*, 1999). In the same study, severe fibrosis associated with portal hypertension was more frequent in certain families and absent in others although they are living in the same conditions (Mohamed-Ali *et al*, 1999). The progression of the disease was controlled by gene locus (*SM2*) that mapped to chromosome 6q22-q23 (Dessein *et al*, 1999; 2001).

## 2.9 Genetic Polymorphisms and Periportal Fibrosis

Polymorphisms are heritable genetic markers. The majority of human DNA sequence variation is attributed to the single nucleotide polymorphism (SNP), in which there is a change in a single nucleotide, while insertions or deletions of one or more bases, and repeat length polymorphisms (microsatellites) are also found. In normal individual SNP occurs once in every 1000-2000 nucleotides, suggesting that about 10 million SNP are present across the whole genome (Cargill *et al*, 1999; Knight, 2001). Only a small proportion of these polymorphisms are of functional relevance by causing structural alteration of the protein encoded by a gene or by altering neighbouring regions of DNA that control gene regulation. All these changes are of potential value as genetic markers for mapping regions of DNA that determine disease susceptibility (Kwiatkowski, 2000). The pathologies of many infectious diseases are influenced by the profiles of cytokine production in pro-inflammatory (Th1) and anti-inflammatory (Th2) T cells. Differences in cytokine profiles among individuals appear to be at least in part, due to allelic polymorphism within regulatory regions of cytokine gene (Bidwell *et al*, 1999). Many studies have examined the relationship between cytokine gene polymorphism and susceptibility to severity of disease (Henri *et al*, 2002b; Chevillard *et al*, 2002; Jahromi *et al*, 2000; Koch *et al*, 2002).

It had been reported previously by (Chevillard *et al*, 2003) in a study done in Sudanese population of endemic area for *S. mansoni*, that two polymorphisms located in the third intron of the IFN- $\gamma$  gene are associated with PPF. The IFN- $\gamma$  +2109 (A/G) polymorphism is associated with higher risk for developing PPF, whereas the IFN- $\gamma$  +3810 (G/A) polymorphism is associated with less risk for developing PPF. These polymorphisms result in changes in nuclear protein interactions with the intronic regions of the gene,

suggesting that they may modify IFN- $\gamma$  mRNA expression (Chevallard *et al*, 2003). In a different study on the same population, Moukoko *et al*, (2003) found no evidence of association between four polymorphisms of TNF- $\alpha$  gene (TNF- $\alpha$  -376 G/A, -308 G/A, -238 G/A, and +488 G/A) and PPF.

#### 2.10 Regression of Periportal Fibrosis

Reversibility of liver fibrosis after antischistosomal therapy in murine schistosomiasis has been widely reported (Emonard and Grimaud, 1989). In human schistosomiasis, evaluating regression of morbidity is an important component of community-based control programmes and chemotherapy with Praziquantel (PZQ) which is the cornerstone of schistosomiasis control (Vennervald *et al*, 2005). Assessment of the impact of mass treatment with PZQ is usually by determining the prevalence of the infection and presence of PPF (Mohamed-Ali *et al*, 1991). Vennervald *et al*, (2005) evaluated the effects of treatment with PZQ on hepatosplenic morbidity in a cohort of Kenyan school-aged children, and they reported regression of hepatosplenomegaly three years after treatment, and suggesting that exposure to schistosome infection may be a significant factor affecting the outcome of PZQ treatment to reduce the level of hepatosplenic morbidity (Booth *et al*, 2004b). Previous ultrasonographic investigations in Sudan reported a reduction of egg excretion and regression of PPF seven months (Mohamed-Ali *et al*, 1991), twenty-three months after PZQ treatment (Doehring-Schwerdtfeger *et al*, 1990), and after both annual and biennial treatment (Homeida *et al*, 1996). Reports by Homeida group in their studies in Sudan have shown that the factors that control fibrosis regression after PZQ therapy involve age, gender, and grade of fibrosis. Young patients with lower grades of PPF tend to respond more to antischistosomal chemotherapy (Homeida *et al*, 1991; 1996).

## MATERIALS AND METHODS

### **3.1 Study design**

Quasi intervention experimental study was carried out in the population of Um Zakra village

### **3.2 Study area**

Initial survey was conducted to determine area with highest prevalence of intestinal schistosomiasis. Random stool samples were taken from different villages in the Gezira state, and examined for *S. mansoni* eggs. ,base on the result the following villages were selected um zukra ,Kambo Tawilla and kamel Nomak .

The highest prevalence of intestinal schistosomiasis (70 %) was found in Um-Zukra ,it is located in Managil province, Gezira State, central Sudan. The village is about 350 km South of Khartoum (The Capital) and 110 km West of Wad Medani town (Figure 3.1). Gezira and Managil irrigated scheme is about two million acres, cultivated by cotton and other crops, and populated by about 1.5 million. The study area is a known endemic area for *S. mansoni* infection. The population belonging to three main tribes, the Kawahla (80%), in addition to Rawashda and Galeen (20%). The village is surrounded with cultivated area and the canal is only 450 m distance from the centre of the village. There are water pumps (wells) used for drinking water. The other water sources for the domestic uses (washing and bathing) is the canal.

### **3.3 Sample size**

One hundred and seventy four individual Sudanese nationals diagnosed as schistosomiasis patients with active *S. mansoni* infection complicated with different grades of PPF out of this number 100 patient continued throughout this study.

### **3.4 Parasitological Methods and Treatment**

Plastic containers for stool samples were distributed to the villagers according to the house and individual numbers. *S. mansoni* eggs count/gram stool has been done in November 2010 using modified Kato's method and its (Katz *et al*, 1972) on three stool samples collected on every three days before treatment. Egg count was represented as geometric mean.



**Figure 3.1:** Sudan map showing the study area (Um-Zukra village).

### 3.4.1 Procedures for Stool Examination

Kato – Katz technique, cellophane faecal thick smear and its modification (Katz *et al*, 1972) were used in this study to diagnose the study subjects for *S. mansoni* eggs in  $\cong$  one gram faecal materials.

A small amount of faecal material was placed on filter paper or a piece of A4 photocopying paper and a piece of nylon screen was pressed on top so that some of the faeces sieved through the screen and accumulated on top. A flat- sided spatula was scraped across the upper surface of the screen to collect the sieved faeces. A template was placed on the slide and the sieved faeces were added with the spatula so that the hole in the template was completely filled. The spatula was passed over the filled template to remove excess faeces from the edge of the hole. Then template was removed carefully so that a cylinder of faeces was left on the slide. The faecal material was covered with a pre-soaked cellophane strip or a clean glass cover slip. The slide was inverted and the faecal sample was pressed firmly against the hydrophilic cellophane strip or the glass cover slip to spread evenly. The slide was placed on the bench with cellophane or the glass cover slip upwards to enable the evaporation of water while glycerol cleared the faeces. The slide was kept for one or more hours at room temperature to clear the faecal material, prior to microscopic examination. The *S. mansoni* egg count/gram faeces were performed microscopically the field (Um -zakra village) or in the laboratory at Wad Medani, and the egg count is represented as geometric mean.

### **3.4.2 Treatment**

All study subjects were weighed first, and then treated with a single dose of Praziquantel (PZQ) tablets (40 mg / kg body weight), manufactured by: Medochemie LTD, Limassol, Cyprus, Lot. No. E5K020.

### **3.5 Ultrasound Evaluation**

Study subjects (n = 174) were evaluated by ultrasound (SSD 500 echo camera and 3.5- MHz convex probe; Aloka, Amsterdam, the Netherlands) before treatment in May 2011. Hundred seventy subjects were evaluated again in August 2012 by the same sinologist. Only 100 subjects were included in the study because they had completed the planned ultrasound investigations. The degree of periportal fibrosis (PPF) was graded as F 0, F I, F II and F III according to the standardized Cairo classification (Cairo Working Group, 1992) and as reported by many authors (Mohamed-Ali *et al*, 1999; Dittrich *et al*, 1983; Abdel-Wahab, 1989). In brief, liver size, peripheral portal branches (PPBs), the degree of PPF, thickness of PPB wall, spleen size and splenic vein (SV) diameter were assessed. Livers and spleens were measured as previously described (Mohamed-Ali *et al*, 1999). 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. Thickness of secondary (PPB) was observed for all subjects with FI-FIII grade of fibrosis. The above ultrasonographical measurements were reported for each individual enrolled in the study (Figure 3.2). Grade 0 (F0) corresponds to normal liver with no thickening of

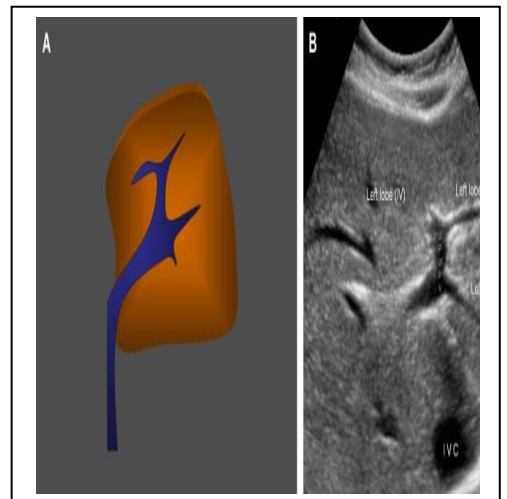
**Figure 3.2:** Reporting of ultrasound evaluation and personal data.





Grade I

Grade II



Grade III

Normal liver

**Figure 3.2:** Ultrasonographic grading of periportal fibrosis, F1, FII and FIII. Narrowing of hepatic veins is obvious in FIII (arrow).

the PPB wall and PPB diameters (outer to outer) ~2-3 mm. Grade I (FI) corresponds to a pattern of small stretches of fibrosis around secondary portal branches and PPB diameters ~ 4 mm. Grade II (FII) 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. Grade III (FIII) shows a thickening of the walls of most PPBs (Figure 3.2).

### **3.6 Clinical Evaluation**

A medical history, personal data (name, sex, age and number of pregnancy for married women), current symptoms, number of malaria attacks/year and physical examination for each subject were performed. Informed consent was obtained from each patient or parents in case of children.

### **3.7 Blood Samples and DNA Preparation**

Five to 10 ml of blood were collected on sodium citrate from 100 study subjects and kept at  $-20^{\circ}\text{C}$  until DNA was extracted using the standard chloroform method (Sambrook *et al*, 1989).

#### **3.7.1 DNA Extraction Procedures**

##### **3.7.2 Extraction Procedures**

Seven to ten ml blood was transferred into a 50 ml falcon tube and then the volume was completed to 45 ml with 0.2% NaCl mixed well, and centrifuged at 3000 rpm for 20 minutes at  $4^{\circ}\text{C}$ . After that the supernatant was discarded and resuspended the pellet with 0.2% NaCl to a volume of 45 ml. The above step was repeated till a clear pellet had appeared.

The cell lysates were digested overnight at  $37^{\circ}\text{C}$  with  $10\ \mu\text{l}$  of  $10\ \text{mg/ml}$  proteinase K,  $1.0\ \text{ml}$  of  $6\ \text{M}$  guanidine chloride and  $300\ \mu\text{l}$  of  $7.5\ \text{M}$   $\text{NH}_4$  acetate. After the digestion was completed the samples were cooled

to room temperature, transferred to another 15ml falcone tube containing 2ml pre-chilled chloroform, vortexed and after one minute centrifuged at 3000 g for 3 minutes. The upper layer from each tube was collected and transferred to a new falcon tube containing 10ml cold absolute ethanol. Tubes were mixed well and kept at -20°C overnight. The tubes were then centrifuged at 3000g for 15minutes, the supernatants were discarded. The pellets were washed with 70% ethanol two times, spinned at 3000g for 15 minutes, and the supernatant were discarded each time and the tubes left inverted into tissue papers for at least one hour. The DNA pellet was resuspended in 200µl ddH<sub>2</sub>O. The DNA was allowed to dissolve for two days at 4 °C before quantification. The optical densities of DNA samples were performed in 1:50 dilution (150 µl total volume) using nano drop No. 02 1998 0317.

### 3.8 Polymerase Chain Reaction (PCR)

DNA samples (n = 100) were amplified by PCR machine (advance primus96, AOAD27110/07from PeQ lab ,Germany ) in a 20µl reaction. Three primers for TGF- 1 +869 T/C, IL-1B -31T/C, IFN-γ rs2069705C/T were investigated in this study. Primers usually come dry and were resuspended in 100pmol/µl as stock according to the manufacturer specifications for each primer.

TGF-1 Forward primer 5'-TTCCCTCGAGGCCCTCCTA-3'

Reverse primer 5'- GCCGCAGCTTGGACAGGATC3'

For forward primer 100pmol/ µl was dissolved in 231µl sterile distilled water,

100pmol/ µl TGF-1Rev primer dissolved in 191µl sterile distilled water

IL-1 fwd (5'-AGAAGCTTCCACCAATACTC-3'), 100pmol/ µl was dissolve in 201µl Sterile distilled water and 100pmol/ µl dissolved in 174µl for

Rev Primer (5'AGCACCTAGTTGTAAGGAAG-3')

IFN- $\gamma$  rs2069705 polymorphism was generated with use of a Fwd primer rs2069705 F) 5'-

TCCAATGTGCCAAAATAATAATAAA3' and Rev one (Seq.# rs2069705 R) 3'AAGCCCTCCACTCTTTGGTT5'

IFN- $\gamma$  rs2069705 fwd 100pmol/  $\mu$ l was dissolve in 193 $\mu$ l Sterile distilled water and 100pmol/  $\mu$ l dissolve in 213 $\mu$ l for IFN- $\gamma$  rs2069705 Rev .

Working solution of each primer, was prepared in a dilution of 1:10 with sterile distilled water.

Ready loaded master mix Maxime PCR premiMix kit(i-taq) from iNtRON biotechnolgy was use in this study. PCR conditions were first optimized in a total of 20 $\mu$ l, each master mix containing 11.5  $\mu$ l sterile distilled water , 4.0 $\mu$ l Master mix, 1.0  $\mu$ l forward primer 10X (10mM) ,1.0  $\mu$ l reverse primer ,10X (10mM) and 2  $\mu$ l DNA (50ng/ $\mu$ l). The master mix for all samples was mixed by vortexing.

A negative control was included for all experiments in which 2 $\mu$ l of ddH<sub>2</sub>O was added instead of DNA. The condition for TGF amplification was as fallows : initial denaturation at 96°C for 10 min, followed by 30 cycles of denaturation at 94°C for 60 s, annealing at 62°C for 60 s, and extension at 73°C for TGF. PCR Conditions for IL-1B –31T/C was: initial denaturation at 95°C for 1 min, followed by 36 cycles of denaturationat 94°C for 45 s, annealing at 54°C for 50 s, and extension at 72°C for 1 min. IFN $\gamma$  initial denaturation at 95°C for 1 min, followed by 36 cycles of denaturationat 94°C for 45 s, annealing at 54°C for 50 s, and extension at 72°C for 45 sec.

Each PCR reaction was confirmed by electrophoresis, PCR products (5  $\mu$ l) were loaded and run in a 20x25 cm agarose gel (1.5%, Invitrogen, UK) in TBE buffer (0.5X) with electrophoresis of gels set at 120 Volts for 45 minutes.

**The bands were visualized by ultraviolet irradiation after staining the agarose gels with in ethidium bromide; Photographs were taken using Bio Rad gel documentation system .**

RFLP analysis for TGF- was carried out with a mixture of 10 µl of the PCR product, 2.0 µL of 10× NE buffer (50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl<sub>2</sub> and 1 mM dithiothreitol, pH 7.9), 0.2 µl of bovine serum albumin enzyme, 0.25 µl of MspA1-I (20 units/mL) (TakaraShuzo) and 7.55 µl of sterile H<sub>2</sub>O. The mixture was incubated at 37°C overnight. The mixtures of digested product was loaded onto agarose gel (total concentration of 4 %). Electrophoresis was performed at 200 V for 150 min at 37°C using 1× TBE buffer as a tank buffer. Bands were visualized with gel documentation system. The digested fragment length were, , 170 + 30 bp for the T allele and 140 + 60 bp for the C allele variant

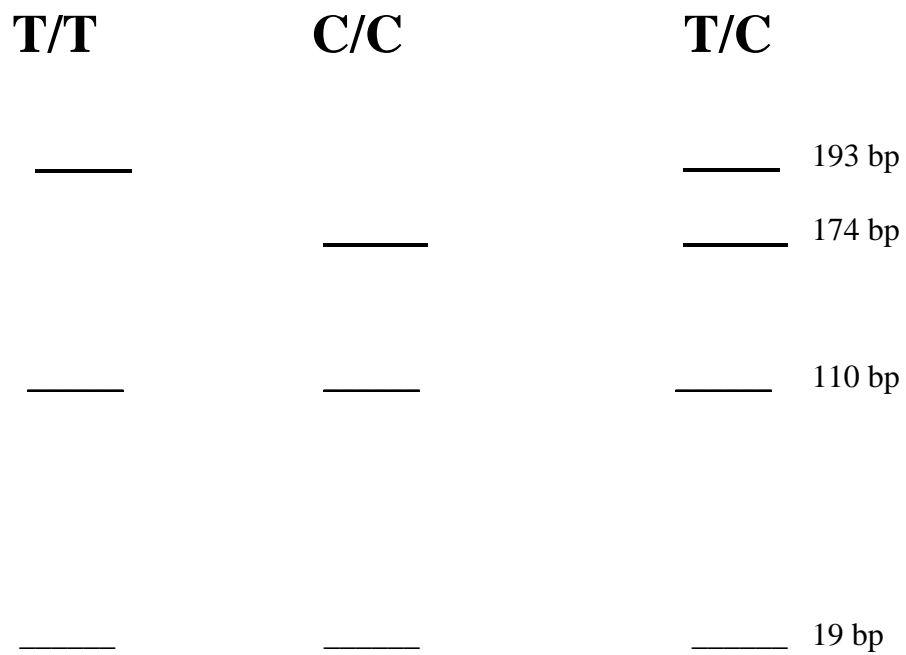
RFLP for IL-1 was carried out using Alu-1 restriction enzyme . In brief, a mixture of 5 µL of PCR product, 2 µL of 10× NE buffer (300 mM NaCl 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 ng/mL bovine serum albumin and 50% glycerol, pH 7.4), 1 µL of Alu I (10 units/µL) (TakaraShuzo) and 12 µL of sterile H<sub>2</sub>O was incubated at 37°C overnight.

The digested product was loaded onto agarose gel (total concentration 4%). Electrophoresis was performed at 200V for 70min using 1× TBE buffer. Bands were visualized by gel documentation system.

RFLP method was used to screen interferon gamma (IFN-γ) rs2069705 polymorphism (C/T). In brief, a mixture of 5 µl of PCR product, 2 µl of 10× NE buffer (300 mM NaCl 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 ng/ul bovine serum albumin and 50% glycerol, pH 7.4), 1 µl of Alu I (10 units/µl) (TakaraShuzo) and 12 µl of sterile H<sub>2</sub>O was incubated at 37°C overnight. The digested product was loaded onto agarose gel (total concentration 4%). Electrophoresis was performed at 200V for 70min using 1× TBE buffer. Bands were visualized by gel documentation system.

### **3.8 Statistical Analysis**

SPSS version 10 (Statistical Package for Social Science), Dynamax and SDS version 2.3 (Sequence Detection Systems) software were used for statistical analysis. Chi- Square was used to compare the two phenotypes (regression and progression) in the study subjects



**Figure 3.4:** IFN- $\gamma$  rs2069705 polymorphism (C/T), the possible genotyping profile using RFLP after *AluI* digestion



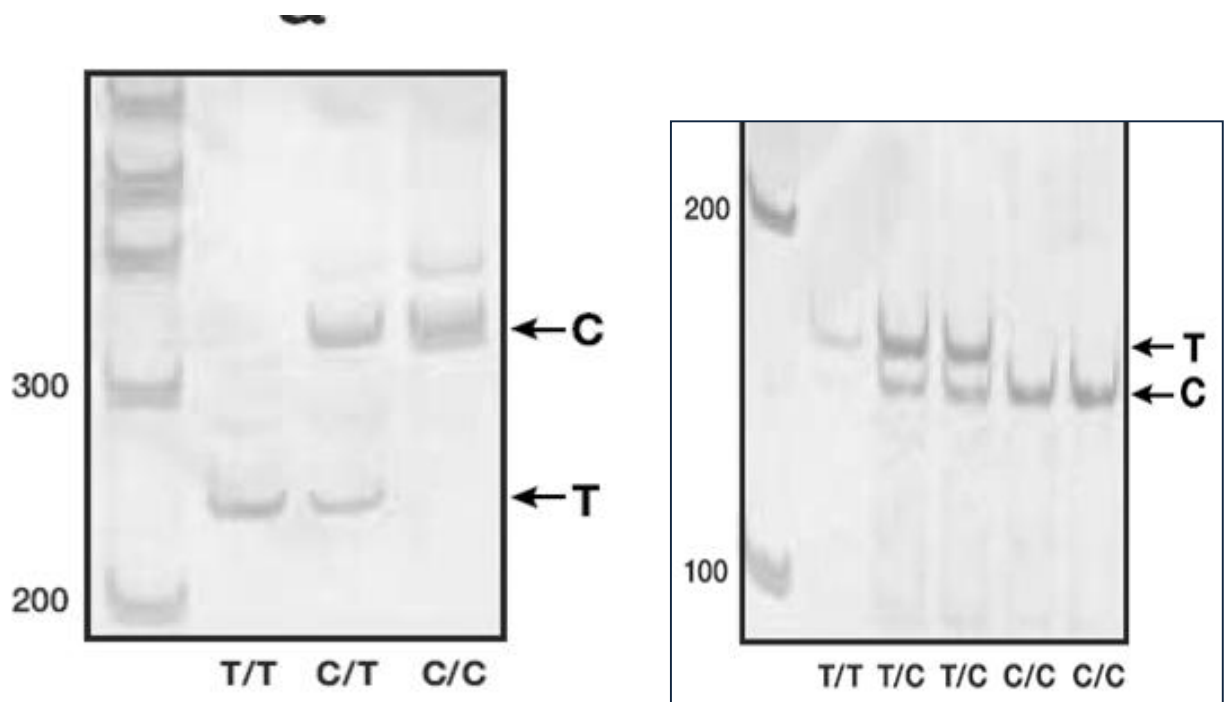


Figure 3.5 Analysis for gene polymorphisms of IL-1 and TGF-1 +869 a: IL-1 -31 T/C genotypes ,b TGF genotypes .

**Table 3.1** Selected single nucleotide polymorphisms (SNPs), and their primer sequences

Markers	Primers sequences
<b>TGF1_fwd</b>	5'-TTCCCTCGAGGCCCTCCTA-3'
<b>TGF1_Rev</b>	5'-GCCGCAGCTTGGACAGGATC-3'
<b>IL1_fwd</b>	5'-AGAAGCTTCCACCAATACTC-3'
<b>IL_Rev</b>	5'-AGCACCTAGTTGTAAGGAAG-3'
<b>IFNg_fwd</b>	TCCAATGTGCCAAAATAATAATAAA
<b>IFNg_Rev</b>	AAGCCCTCCACTCTTTGGTT

**Table 3.2:** Selected single nucleotide polymorphisms (SNPs), their chromosomal and structural location.

Polymorphism (SNP)	Gene	Chromosoma l location	Gene Bank ID	Structural location
C/T	IFN- $\gamma$	12q14	rs2069705	Promoter
T/C	TGF+14869	19q13.2	rs1800470	Coding for protein
T/C	IL-I	2q13-14	rs	Promoter

### 3.9 Ethical approval

Ethical approval for the study was obtained from the ethical committee of State Ministry of Health, Gezira . Sudan

Informed consent was declared to the participants prior to start of the study

## RESULTS

### 4.1 Fibrosis Grades Before and After Treatment

Ultrasound examination of untreated *S. mansoni* infected patient revealed that 64%,20%,and 8% patients had mild (FI),moderate (FII) and severe (FIII) PPF, respectively ; no normal pattern (F0)was observed .After treatment . There was a significant shift towards lower PPF grades and 38% cases reversed to normal (F0) state (Table 4.1).

While the proportions of patients with FI, F II and F III after therapy was 28 %,15 and 19 respectively. (Table 4.1).

### 4.2 Prognosis of the Disease

As seen from table 4.2 ,46 % PPF regressed to lower grades after PZQ treatment ;34from FI to F0,4 from FII to F0,6 from FII to FI and 2 from FIII to FII.

The inter-movement of PPF lower grades towards higher grades was recorded in 20 patients (table 4.2) in which PPF was progressed in 7 patients from (F I) to (F II), one patient was progressed from F I to F III, and 12 patients were progress from FII to FIII. The percentage of the patients in whom PPF was regressed from higher grades of fibrosis to lower ones (reversibility) was 46 % and those in whom PPF was progressed from lower grades of fibrosis to higher ones was 20 % while in 34 % of the patients the PPF was stable.

**Table 4.1:** PPF grades before, and 12 months after treatment with PZQ (40 mg / kg body weight) in 100 study subjects

$$P = 0.0001$$

**Table.4.2:** PPF grades 12 months after treatment with PZQ (40 mg / kg body weight) in 100 study subjects

Period	Fibrosis grades				
	F 0 (%)	F I (%)	F II (%)	F III (%)	Total
Before treatment	0 (0)	64(64.0%)	28(28.0)	8(8.0%)	(100)
After treatment	38(38.0%)	28(28.0%)	15(15.0%)	19(19.0%)	(100)

Fibrosis grades	Phenotype		
	Regression	Progression	Stable
<b>F0</b>	<b>38 (38)%</b>	0(0%)	0(0%)
<b>FI</b>	<b>6(6%)</b>	0(0%)	<b>22(22)%</b>
<b>FII</b>	<b>2(2%)</b>	<b>7(7%)</b>	<b>6(6%)</b>
<b>FIII</b>	0(0%)	<b>13(13%)</b>	<b>6(6%)</b>
<b>Total</b>	46	<b>20</b>	<b>34</b>

The change in PPF after PRQ treatment in relation to gender can be seen in table 4.3 .In this study the total number of male to female was 48and 52 respectively with ratioof about 1:1

The number of male with regression, progression and stable was(19) 39.6%, (15)31.3% and(14) 29.1% respectively while females with

regression, progression and stable was (22) 42.3%,(3) 5.8% and(27) 51.9% respectively. PPF was relatively better in females and youngest patients (16-20 years old ) compared to males and other age groups .  
( $P = 0.002$ ) (table 4.3)(table 4.4)

**Table 4.3:** Response to PZQ treatment in males and females study subjects.

<b>Sex.</b>	<b>Regression of PPF (%)</b>	<b>Progression of PPF (%)</b>	<b>Stability of PPF (%)</b>	<b>Total (%)</b>
<b>Male</b>	<b>19 39.6%</b>	<b>15 31.2%</b>	<b>14 29.2%</b>	<b>48 100%</b>
<b>Female</b>	<b>22 42.3%</b>	<b>3 5.8%</b>	<b>27 51.9%</b>	<b>52 100%</b>

$P = 0.002$

4.4 Changes in PPF in response to treatment with PZQ according to the age and gender after 12 month .

<b>Age group in years</b>	<b>Regression of PPF In female</b>	<b>Regression of PPF in male</b>	<b>Stability of PPF In female</b>	<b>Stability of PPF in male</b>	<b>Progression of PPF in female</b>	<b>Progression of PPF in male</b>
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16-20	7	5	7	5	0	3
21-25	5	4	2	0	1	2
26-30	3	2	4	2	1	3
31-35	4	4	4	2	1	3
36-40	3	3	2	1	1	2
41-50	2	4	4	1	1	2
Sub total	24	22	23	11	5	15
<b>Total</b>	<b>46</b>		<b>34</b>		<b>20</b>	

### **4.3. Genotyping of Single Nucleotide Polymorphisms (SNPs)**

Three polymorphisms (IFN- $\gamma$  rs2069705 (C/T), TGF- 1 +869 and IL-1 (31 T/C) have been screened in this study (Table 3.1).

#### **4.3.1 Screening of the Polymorphism IFN- $\gamma$ rs2069705 (C/T) in PPF within study subjects.**

The genotyping frequency of IFN- $\gamma$  rs2069705 (C/T) in 90 study subjects was (42) 46.7% for CT, (11)12.2% for CC and(37) 41.1 for TT. The ancestral allele frequency (C) of this polymorphism was 35.5 % (Table 4.4).

When the different genotyping of SNP IFN- $\gamma$  rs2069705 (C/T) were crossed tabulated with fibrosis grades before treatment (table 4.5) it was shown that, the frequency of CC homozygous was(7) 63.6 % (2),18.2%.(2)18.2% in patients with fibrosis grade I, grade II and grade III respectively. while the genotyping frequency of CT heterozygous and TT homozygous together was(54) 65.8 % in patients with fibrosis grade one (F I) , (22)27.8 % in patients with fibrosis grade II (F II), and(5) 6.4% in patients with fibrosis grade III. With no association between IFN- $\gamma$  (C/T) polymorphism and fibrosis grades (P = 0.352).

Table 4.6 showed the different genotyping of SNP IFN-G rs2069705 (C/T) when crossed tabulated with disease prognosis (regression,

progression and stable phenotypes). The genotyping frequency of CT heterozygous and TT homozygous together was (33) 41.8% in patients with regression phenotype and was (23)29.1% in those with progression phenotype. The genotyping frequency of homozygous CC within regression ,progression phenotype and stable was (3)27.3% and (3)27.3 and 5(45.4)respectively. No association was reported between SNP IFN-G rs2069705 (C/T) and disease prognosis ( $P = 0.510$ )

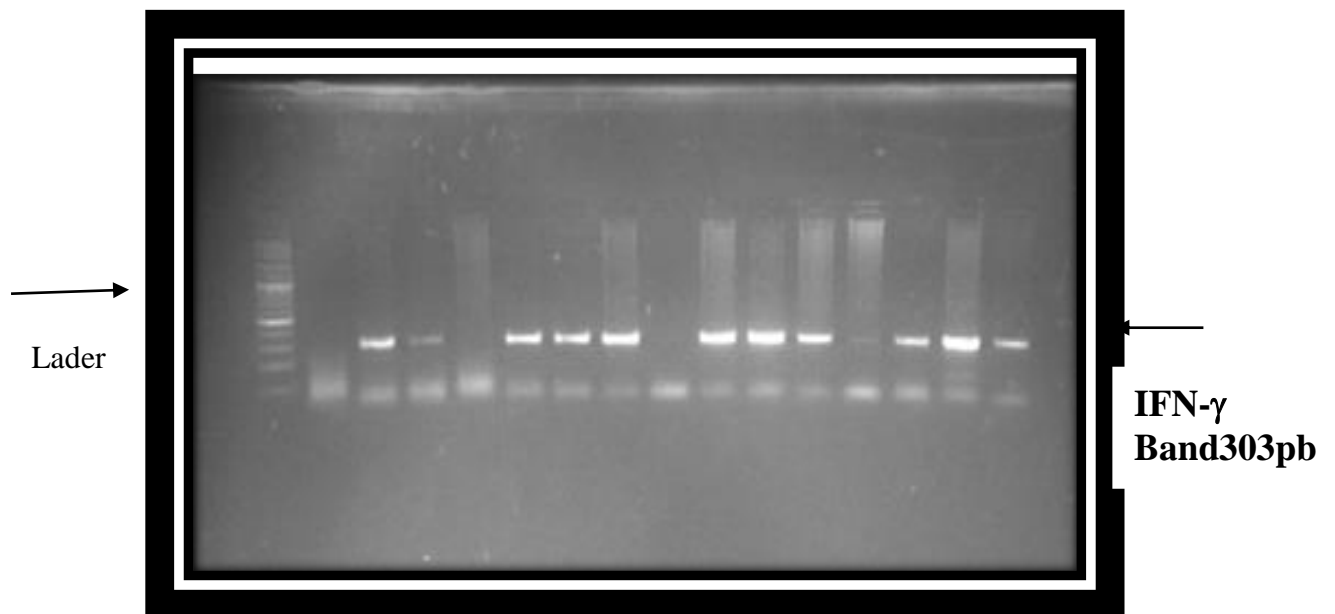
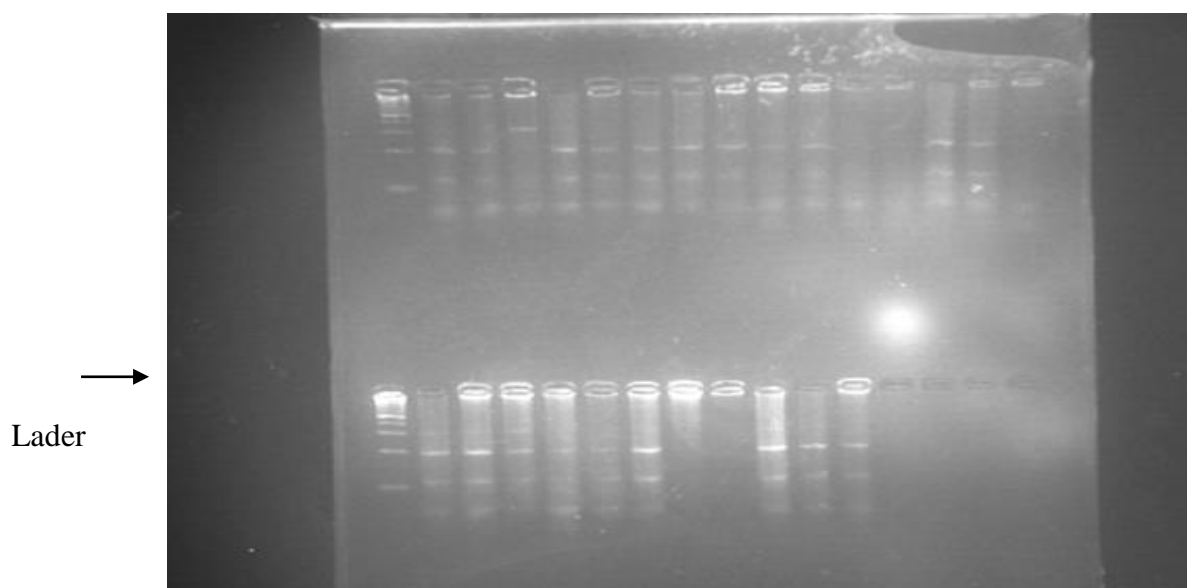


Figure4.1 PCR for SNP IFN-G rs2069705 (C/T)





TC TC CCTC TT TCTC TC

Figure 4.2: Genotyping of IFN- $\gamma$  rs2069705 polymorphism (C/T) in

Genotyping of IFN- $\gamma$ (C/T)	number	Frequency	Allele frequencies
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DNA samples of Sudanese patients infected with *S. mansoni* showing T/T, C/C homozygous and T/C heterozygo.

**Table 4.5:** The frequency of different genotypes of IFN- $\gamma$  rs2069705) in study subjects

<b>C/T of INF</b>	<b>42</b>	<b>46.7%</b>	<b>Allele C</b> <b>35.5</b>
<b>C/C</b>	<b>11</b>	<b>12.2%</b>	
<b>T/T</b>	<b>37</b>	<b>41.1%</b>	
<b>Total</b>	<b>90</b>	<b>100%</b>	

P value=0.352

**Table 4.6:** The frequency of different genotypes of SNP IFN rs2069705 (C/T) when cross-tabulated with the fibrosis grades in 90 study subjects

**Table 4.7:** The frequency of different genotypes of SNP IFN-

<b>Fibrosis grade</b>	<b>C/C (%)</b>	<b>C/T or T/T (%)</b>	<b>Total (%)</b>
<b>FI</b>	<b>7 (63.6%)</b>	<b>52(65.8%)</b>	<b>59(65.5%)</b>
<b>FII</b>	<b>2(18.2%)</b>	<b>22 (27.8%)</b>	<b>24(26.7%)</b>
<b>FIII</b>	<b>2 ( 18.2%)</b>	<b>5 (6.4%)</b>	<b>7(7.8%)</b>
<b>Total</b>	<b>11 (100%)</b>	<b>79 (100%)</b>	<b>90 (100%)</b>

rs2069705

(C/T) when cross-tabulated with the disease prognosis in 90

study subjects.

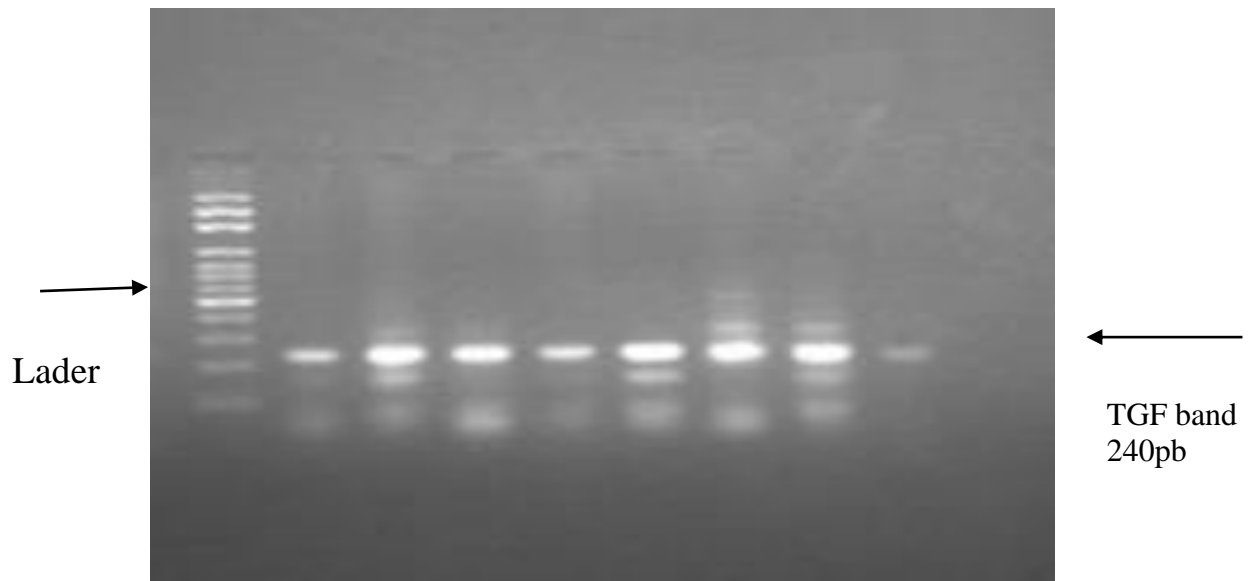
<b>Disease prognosis</b>	<b>C/C (%)</b>	<b>C/T or T/T (%)</b>	<b>Total</b>
<b>Regression</b>	<b>3 (27.3%)</b>	<b>33 (41.8%)</b>	<b>36(40%)</b>
<b>Progression</b>	<b>3( 27.3%)</b>	<b>23( 29.1%)</b>	<b>26(28.9%)</b>
<b>Stable</b>	<b>5(45.4%)</b>	23(29.1%)	<b>28(31.1%)</b>
<b>Total</b>	11(100%)	79(100%)	<b>90 (100%)</b>

*p* value =0.510

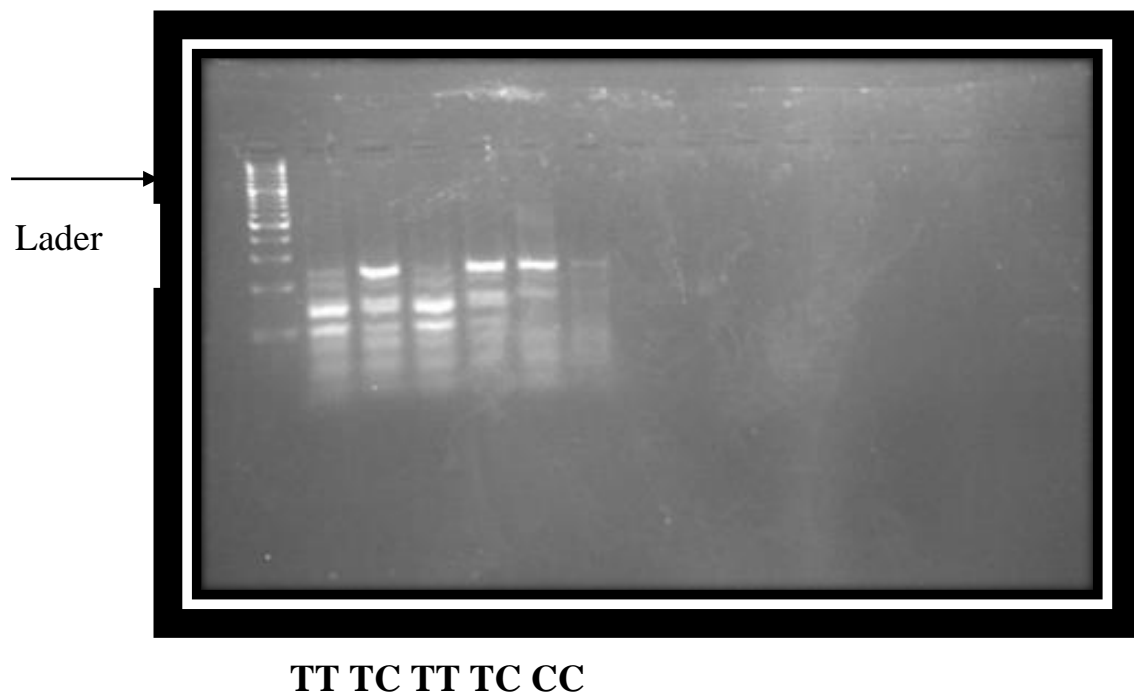
### **4.3.2 Screening of TGF+14869 polymorphism in PPF patient.**

The genotyping frequency of homozygous CC was (31) 32%, the genotyping frequency of heterozygous TC was (5)5.1%, while TT homozygous frequency was (61)62.9 %. The minor allele frequency of this polymorphism (allele T) was 25.3% (table 4.7). When the different genotypes of this polymorphism were crossed tabulated with fibrosis grades, it was shown that the genotyping frequency of TC heterozygous and TT homozygous together was (48)72.7 % in patients with fibrosis grade (F I) , (14) 21.2% in patients with fibrosis grade FII ,and (4)6.1% in patients with fibrosis grade (F III) . The homozygous CC genotyping frequency of this polymorphism was(14) 45.2 %,(14) 45.2%, and (3)9.6% in patients with fibrosis grade one , grade two and grade three respectively (table 4.8). statistically significant different between CC genotype and TC and TT genotypes group and fibrosis grades ( $p=0.029$ ).

The genotyping frequency of TC heterozygous and TT homozygous together was(33) 50.2% in patients with regression phenotype and(10) 15.2 % in those with progression phenotype and (23)34% were stable . The genotyping frequency of CC homozygous was (10)32.3% in patients with regression phenotype and (11)35.5% in those with progression phenotype, while (10)32.2% of patients were stable. with no significant relation between different genotypes and prognosis of disease ( $p=0.062$ ).



**Figure4.3 :- PCR product for TGF+ 14869 T/C polymorphism**



**Figure 4.4 :Table 4.4: the genotyping frequency of TGF+ 14869 T/C polymorphismwith in the Study subject .**

**Table 4.7:** The frequency of different genotypes of SNPTGF+14869 (T/C ) in 97 study subjects

Genotyping of Disease TGF (T/C) prognosis	Frequency C/C (%)	C/T or T/T (%)	Allele frequencies	
			Allele C	Total
T/C	61	(62.9%)	Allele C	71.7 %
T/T	5	(5.1%)	Allele T	25.3 %
C/C	31	(32%)		
<b>Total</b>	<b>97</b>	<b>(100%)</b>		

**Table 4.8:** The frequency of different genotypes of SNP TGF+14869 (T/C ) when cross-tabulated with the disease fibrosis in 97 study subject

Fibrosis grade	C/C (%)	T/C or T/T (%)	Total (%)
<b>FI</b>	<b>14(45.2%)</b>	<b>48 (72.7%)</b>	62(63.9%)
<b>FII</b>	<b>14(45.2%)</b>	<b>14(21.2%)</b>	<b>28(28.9%)</b>
<b>FIII</b>	<b>3 (9.6%)</b>	<b>4(6.1%)</b>	7(7.2%)
<b>Total</b>	<b>31(100%)</b>	<b>66(100%)</b>	<b>(100%)</b>

*P* value =0.029

**Table 4.9:** The frequency of different genotypes of SNP TGF+14869 (T/C) when cross-tabulated with the disease prognosis in 97 study subject

<b>Regression</b>	<b>10(32.3%)</b>	<b>33 (50%)</b>	<b>43(44.3%)</b>
<b>Progression</b>	<b>11 (35.5%)</b>	<b>10(15.2%)</b>	21(21.7%)
<b>Stable</b>	<b>10(32.3%)</b>	23(34.8%)	33(34%)
<b>Total</b>	31(100%)	66(100%)	<b>97 (100%)</b>

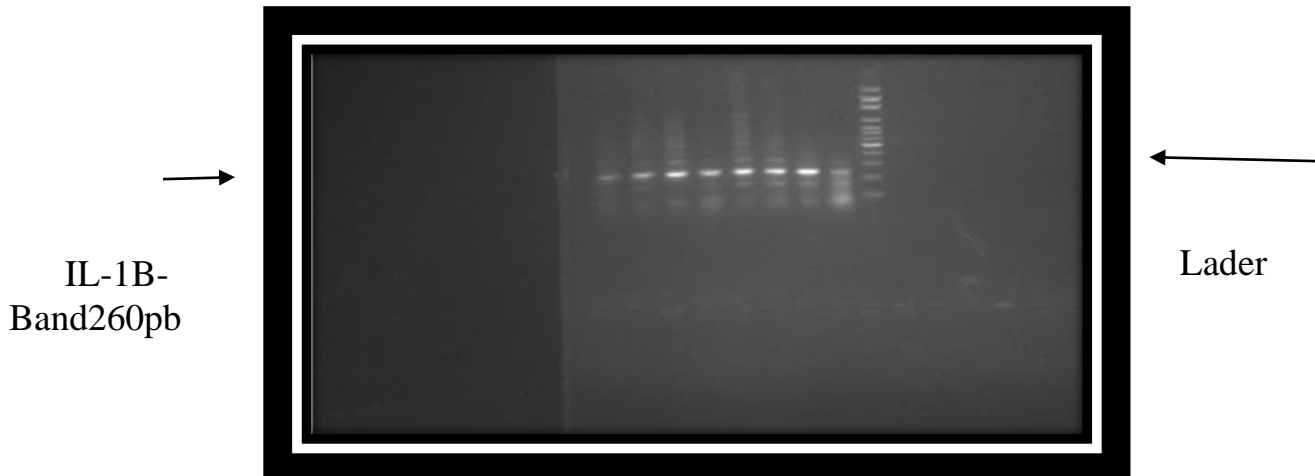
**P value =0.062**

### **4.3.3 Screening of the polymorphism *IL-1B* (-31 T/C ) in PPF within *S. mansoni* patients**

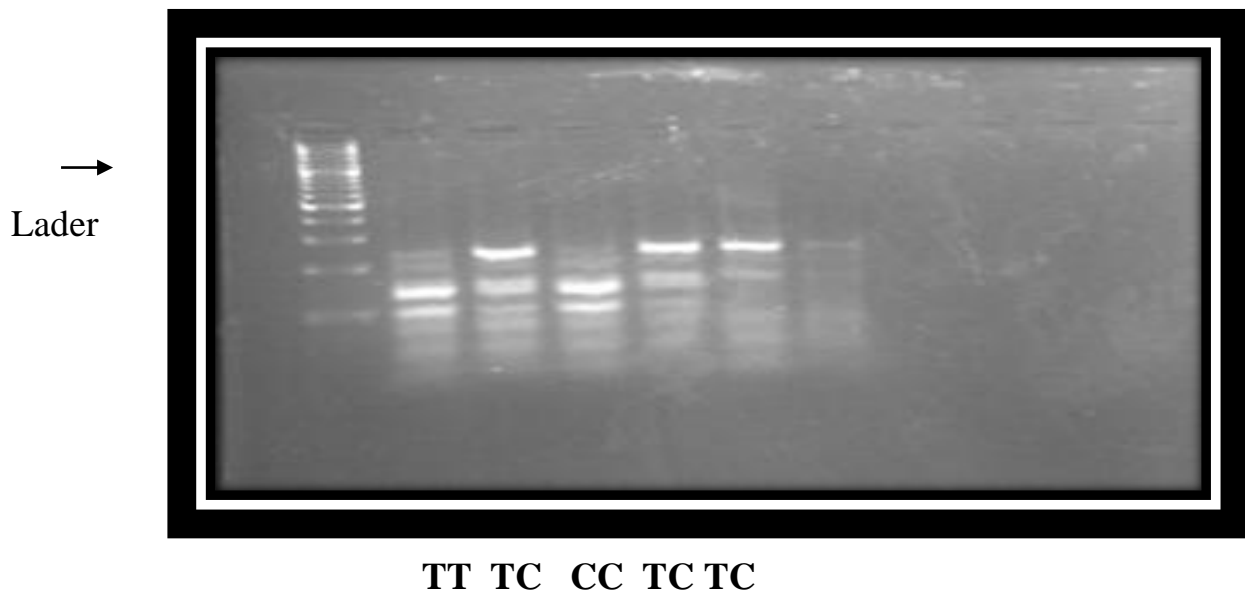
The genotyping frequency of this polymorphism in 68 study subjects were, (67.6)46%,(11.8) 8% , and (20.6)14% for the heterozygous TC, homozygous TT, and homozygous respectively. the frequency of ancestral allele was (21.7%) (table 4.10). The genotyping frequency of TC heterozygous and TT homozygous together in patients with fibrosis grade one (F I), FII, and FIII was(37) 68.5,(12) 22.2 % , and(5) 9.3% respectively. The homozygous CC genotyping frequency was(5) 35.3 % in patient with fibrosis grade one (F I),(8) 57.1% in patients with fibrosis grade two, and (1)7.2% in patients with fibrosis grade three (F III) (table 4.11). The difference between CC genotype and TC/and TT genotype among the fibrosis grades was statistically significant ( $p=0.037$ ).

Table 4.12 shows the different genotypes of IL-I 31 T/C when crossed tabulated with disease prognosis (regression and progression phenotypes). The genotyping frequency of TC heterozygous and TT homozygous together was 49.2% in patients with regression phenotype and was 16.6 % in those with progression phenotype. The genotyping frequency of CC homozygous was 7.1% in patients with regression phenotype and was 71.4 % in those with progression phenotype. The

difference between CC genotype and TC/and TT genotype among disease prognosis was statistically significant ( $p=0.0001$ ).



**Figure 4.5 PCR For IL-1B-31T/C**



**Figure 4.6 the genotyping frequency of IL-1B-31T/C**



**polymorphismwith in the Study subject**

**Table 4.10:** The frequency of different genotypes of SNP

IL -131 (T/C ) in 68 study subjects

<b>Genotype</b>	<b>Frequency</b>	<b>(%)</b>	<b>Allele frequencies</b>
<b>T/C</b>	<b>46</b>	<b>(67.6%)</b>	<b>Allele C 21.7 %</b>
<b>T/T</b>	<b>8</b>	<b>(11.8%)</b>	<b>Allele T 46.3 %</b>
<b>C/C</b>	<b>14</b>	<b>(20.6%)</b>	
<b>Total</b>	<b>68</b>	<b>(100%)</b>	

**Table 4.11.:** The frequency of different genotypes of SNP IL -1B -31

<b>Fibrosis grade</b>	<b>C/C (%)</b>	<b>T/C or T/T (%)</b>	<b>Total (%)</b>

(T/C) when cross-tabulated with the disease prognosis

<b>FI</b>	5(35.7%)	37 (68.5%)	42(61.8%)
<b>FII</b>	<b>8(57.1%)</b>	<b>12(22.2%)</b>	<b>20(29.4%)</b>
<b>FIII</b>	1(7.2%)	5(9.3%)	6(8.8%)
<b>Total</b>	<b>14(100%)</b>	54(100%)	<b>68(100%)</b>

*P* value =0.037

**Table 4.12:** The frequency of different genotypes of SNP IL -1B-31(T/C) when cross-tabulated with the disease prognosis in study subject.

<b>Disease prognosis</b>	<b>C/C (%)</b>	<b>C/T or T/T (%)</b>	<b>Total</b>
<b>Regression</b>	<b>1(7.1%)</b>	<b>32 (49.2%)</b>	33(41.8%)
<b>Progression</b>	<b>10 (71.4%)</b>	<b>11(16.9%)</b>	21(26.6%)
<b>Stable</b>	3(21.5%)	<b>22(33.9%)</b>	25(31.6%)
<b>Total</b>	14(100%)	65(100%)	79(100%)

*P* value =0.001

## DISCUSSION

In this study, the percentage of regression in patients with F II to FI was 40% and from FIII to FII decreased was 46 after 12 months of treatment. In contrast 20 %; FI to FII 35% from FI, FII to FIII was 65% move to higher grades. This finding was consistent with the previous studies done in Sudan, which reported regression of PPF after seven months, twenty three months and after both annual and biennial Praziquantel therapy (Mohamed-Ali *et al*, 1991; Kheir *et al*, 2000; Homeida *et al*, 1996).

Praziquantel treatment was found to decrease the parasitic load by killing the adult schistosomes and reported to kill and dislodge the eggs from the portal tracts. This step arrested further PPF formation and participates PPF resolution with the probability that dead ova becomes less immunogenic. The dose of praziquantel and the post treatment assessment can constitute important factors in lessening the severity of PPF as previously reported (Rahoud, *et al*. 2010).

In this study, the regression of PPF differed according to the severity PPF grades with better regression in low grades and youth than high grades and older patients this finding is consistent with the finding of Rahoud, *et al.*, (2010). Despite that, some of the PPF cases remained stable. In this aspect, clearance of the parasites from the body should be assured, i.e. whether it's due to failure of cure or re-infection. Time that relapsed after treatment and genetic factors may play roles in the progression of PPF it possible that role of immune response, if it plays synergistic role, was not adequate or effective to work with the drug for clearance of infection.

The regression of PPF in this study was found to be better among the females compared to the males and such a finding was reported

previously in Sudan (Homeida *et al*, 1991)). The physiology of the female (hormonal or others) probably contributed significantly to this outcome. Moreover, females may be harbouring lower parasitic load than the males; a situation that can be resolved by the egg count.

The stability of PPF in 34% among the study subject explained either those patients were genetically susceptible to develop severe PPF or they did not respond adequately to treatment. The large number of patients in the present study in whom PPF was stable does not mean that the pathology of the disease had been stopped, but we can assume that those patients may need more time (> 12 months) in order either the disease reverses or may progress, or the Praziquantel therapy should be repeated as reported previously (Homeida, *et al*., 1996).

The present findings showed that PPF progressed significantly more in males (31.2%) than in females (5.8%). Similarly previous studies by Mohamed *et al*, (1999) showed that intensity of *S. mansoni* infections and prevalence of Schistosoma PPF were significantly higher in males than females. Because males do most of the farming activities in our study population, the observed high prevalence of PPF is probably related to sex-related behavioural and occupational differences of exposures to potentially infected water bodies, which put males at higher risk of acquiring *S. mansoni* infection, as well as that female reproductive hormones have an antifibrogenic effect (Xu *et al*, 2002), while male reproductive hormones have a fibrogenic effect (Colborn *et al*, 1993).

Three polymorphisms (IFN- $\gamma$  rs2069705 (C/T), TGF-1 +869 and IL-1 (31 T/C) were screened in this study.

The selection of these genes (IFN- $\gamma$ , TGF-1, and IL-1B 31) based on their cytokines production which regulate the extracellular matrix protein production as reported by many authors (Henri *et al*, 2002b; Chevillard *et al*, 2002; Jahromi *et al*, 2000; Koch *et al*, 2002; Moukoko *et al*, 2003).

The localization of IFN- $\gamma$  gene close to SM2 in the 6q22-23 chromosomal region which was showed to be a strong correlate to PPF in intestinal schistosomiasis (El-Wali, 2002). IFN- $\gamma$  is a key regulator of the development and functions of the immune system. It plays a major role in immune defence against infections for various human pathogens (Henri *et al*, 2002a; 2002b). It was reported as a strong antifibrogenic cytokine that inhibits the production of extracellular matrix proteins (ECMP) by stellate cells and increases the collagenase activity of the liver by stimulating metalloprotease (MP) synthesis and by inhibiting the synthesis of tissue inhibitors of MP (TIMP) (Doncan *et al*, 1985; Tamai *et al*, 1995)

In regions endemic for *S. mansoni*, 5–10% of infected subjects develop a severe hepatic disease characterized by PPF and portal hypertension. Previous studies in humans have suggested that difference in IFN- $\gamma$  production could account, at least in part, for the different rate of fibrosis progression in populations in endemic areas (Henri *et. al*, 2002). Putative sequence changes in noncoding regions of a gene may influence gene expression due to the creation or alteration of DNA-binding sites for transcription factors. Several reports have suggested that specific regulatory elements located in the first, second, and third intron of the IFN- $\gamma$  gene bind nuclear proteins that may have a role in the control of IFN- $\gamma$  transcription (Young and Ghosh. 1997.) This led us to investigate whether any SNPs in the IFN- $\gamma$  gene could be associated with severe fibrosis.

In this study, we investigated whether IFN- $\gamma$  (rs2069705 C/T) gene polymorphism could contribute to disease prognosis in schistosomiasis. As it was found that the minor Allele frequency for this polymorphisms (allele C) was 37.5% . Although, there is different between in genotyping frequency between CC homozygous and TT / TC

heterozygous among fibrosis grades and disease prognosis a significant association between this polymorphism and PPF grades and/or disease prognosis was not detected ( $p > 0.05$ ).

Evidence of association between two polymorphisms within IFN- $\gamma$  gene and PPF development was reported previously in an area endemic with *S.mansoni* infection in Sudanese population. The IFN- $\gamma$  +2109 (A/G) polymorphism was associated with higher risk for developing PPF, whereas the IFN- $\gamma$  +3810 (G/A) polymorphism was associated with less risk for developing PPF. These polymorphisms result in changes in nuclear protein interactions with the intronic regions of the gene, suggesting that they may modify IFN- $\gamma$  mRNA expression (Henri et al., 2002)

A few studies have associated alterations of the IFN- $\gamma$  pathway with diabetes type 1, arthritis, lupus, multiple sclerosis, hepatitis B infection, malaria, and bacterial infection.

Thus, genetic alterations in the IFN- $\gamma$  gene or the IFN- $\gamma$  signal transduction pathway may result in an altered clinical course of disease progression (Henri et al., 2002)

TGF-1 is a pluripotent cytokine that promotes hepatic fibrogenesis by stimulating the synthesis of extracellular matrix (ECM), inhibiting the degradation of ECM, and activation of hepatic stellate cells. Indeed, the plasma TGF-1 concentration is increased in patients with chronic hepatitis, and correlated with the degree of liver fibrosis (Murawaki et al., 1998).

The present study, attempted to evaluate whether the polymorphism (TGF-1 +869 T/C) has an association to PPF development induced by *S. mansoni* infection in selected Sudanese population. The result showed that there was no significant association between this polymorphism and disease prognosis but we found that TGF-1 +869

T/C T/T homozygous and C/T heterozygous genotypes were more frequent (72.7%,  $P = 0.029$ ) in subjects with low grade of fibrosis (F I). In agreement with Suzuki and co-workers we found no significant association between TGF-1+869T/C with the progression of liver fibrosis in study subject ( $P = 0.062$ ). There are several studies about the association of TGF-1 gene polymorphisms with the progression of liver fibrosis in patients with HCV related chronic liver disease, but the results are controversial. Powell *et al.* (2000) have shown that the TGF-1 +915G/G homozygote with high transcriptional activity was more often in stage III/IV in Australian HCV-related chronic liver disease patients, whereas TGF-1 -509C/T and +869T/C did not differ among the stages of fibrosis. In contrast with Powell's data, Wang *et al.* (2003) have shown that the TGF-1 +869 T carrier and +915 G/C heterozygote are more often in stage III/IV in German HCV patients. Suzuki *et al.* (2003) have shown no association of TGF-1 +869T/C polymorphism with the progression of liver fibrosis in Japanese HCV patients. (Suzuki *et al.*, 2003)

It seems rather difficult to pin point the reasons for such differences. However, race, probes adopted, concurrent diseases and health status may act as contributing factors.

IL-1B is a key cytokine in the inflammatory response and its biological activities promote various diseases against microbial threats including chronic hepatitis.

This cytokine participates to the generation of systemic and local responses to infection, injury and immunologic challenges by generating fever, activating lymphocytes and by promoting leukocyte infiltration at sites of injury or infection (Dinarello, 1996).

IL-1B stimulates the progression of liver fibrosis. In fact, activated hepatic stellate cells (HSC) have been clearly recognized as the main



effectors of collagen production in the liver (Iredale, 2008) the pro-fibrotic role of other cell types within the liver, such as macrophages and Kupffer cells, involved in the modulation of the inflammatory process linked to fibrosis development, have the capacity to release a broad panel of pro-inflammatory cytokines implicated in HSC regulation, such as tumour necrosis factor  $\alpha$ , interleukin 6 and interleukin 1 $\beta$  (IL-1 $\beta$ ).

Serum levels and hepatic mRNA levels of IL-1 were reported to be elevated in patients with chronic hepatitis C (Tilg et al., 1992; Gramantieri et al., 1999).

The IL-1 gene family on chromosome 2q13-14 encodes IL-1 $\beta$  by the IL1B gene. These genes are highly polymorphic and several polymorphisms have been reported. In the IL1B gene two polymorphisms have been described in the promoter region giving origin to transitions at positions -511 (-511 C>T) and -31 (-31 T>C).

The polymorphisms -511C/T and -31T/C were reported to be located in the promoter region of IL-1 and they showed to be in total linkage disequilibrium (El-Omar *et al.*, 2000). The IL-1B -31 C allele has higher transcriptional activity than the IL-1-31 T allele ((Bataller *et al.*, 2003) suggesting -31C allele carriers may over produce the IL-1 protein, resulting in a more aggressive inflammation and in a rapid progression of liver fibrosis.

In the present study, we evaluated whether the same polymorphism (ILB-1 -31 T/C) has an association with PPF induced by *S. mansoni* infection. We found that (ILB-1 -31 T/C) TT homozygous and CT heterozygous genotypes were significantly more frequent in subjects with low grade of fibrosis (F I) compared with homozygous CC ( $p = 0.037$ , and on the other hand (ILB-1 -31 T/C) TT homozygous and CT

heterozygous, were statistically significant more frequent (49.2%,  $P = 0.0001$ ) in subjects with regression phenotype.

Takamatsu *et al.* (2000) have reported that the carriers of the ILB-1 31 T/C were significantly more common in Japanese patients with alcoholic cirrhosis than in those with non-cirrhotic alcoholic liver disease. By contrast, Bahr *et al.* (2003) have reported that genotype distribution was similar between HCV-induced chronic hepatitis and cirrhosis in the German population. Tanaka *et al.* (2003) have also reported that IL-1 – 511C/T genotype distribute did not differ between F0-F2 and F3-F4 in HCV-related chronic liver disease in the Japanese population. Fontanini and his co workers found that IL-1 $\beta$  pro inflammatory polymorphisms inflammatory activity appears to be, at least in part, more pronounced in males while appears blunted in females.(Fontanini et al., 2010).

Reports regarding chronic liver diseases, however, have given conflicting results. A first, likely explanation resides in ethnic related differences in the allelic frequencies. Small sample size and biases in the selection of patients and controls may also have been important sources of variability the possibility that some of the discrepancies in the above mentioned studies may be gender related has not been evaluated so far.

## References

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## Appendix

DNA Extraction reagents

Red Blood Cells Lyses Buffer (0.2 % NaCl).

WBCs Lyses Buffer

10 mM Tris HCl-

10 mM EDTA Proteinase-K (16.5 mg/ml)

10 % SDS

6 M NaCl

### PCR Reagents

Primer ( Attached paper)

Master Mix ( Attached paper )

Preparation of buffer

Tris-EDTA (TE) 20:5 mM 2.6.1.5 Preparation of TBE 5X

1- Tris base (Trisma) or Tris Amino (Euromedex)

54 g

2- Boric acid (Euromedex)

27.5 g

3- EDTA (Sigma)

20 ml

4- Dissolve the above components in 1000 ml Distilled water

5- To prepare TBE 0.5X, take 100 ml of TBE 5X and add it to 900 ml distilled water.

### **Preparation of Agarose gel (1.5 %)**

1- Dissolve 4.5 g of agarose (Invitrogen, UK) in 300 ml TBE 0.5X

2- Put it in the Microwave (Triple Distribution System, LG) to complete dissolving.

3- Pour the dissolved agarose in a gel apparatus or chamber and leave it at room temperature for 20 minutes to solidify.

### **PCR Reagents for one Mix**

Sterile		distilled	water
11.5 $\mu$ l			
MgCl <sub>2</sub>		(25	mM)
0.5 $\mu$ l			
Forward	primer	10X	(10mM)
1 $\mu$ l			
Reverse	primer	10X	(10mM)
1 $\mu$ l			
Master			mix
4			
DNA			(25ng/ $\mu$ l)
2 $\mu$ l			

Total

20  $\mu$ l