ROLES OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE PROMOTER REGIONS OF TUMOUR NECROSIS FACTOR-α AND INTERLEUKINE-1O GENES IN *SCHISTOSOMA HAEMATOBIUM* INFECTION SUSCEPTIBILITY

Amos Marume

213524974

A thesis submitted to the University of KwaZulu Natal, School of Laboratory Medicine and Medical Sciences, College of Health Science, in fulfilment of the requirements for the degree of Doctor of Philosophy in Medical Microbiology (Immunogenetics and Immunoepidemiology)

Durban

November 2020

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This is to attest that contents outlined in this thesis are the original research work done and reported by the author (Amos Marume). The research work detailed in this thesis has not been previously submitted to any institution for award of a degree or diploma. The use of other researchers/scientist' work in the text has been acknowledged accordingly.

Signature….. [redacted] **……Date……..22/11/2019.....**

Amos Marume, Registration Number……..213524974…

As the candidate's supervisors, we have approved this thesis for submission.

1. **Professor Takafira Mduluza (Main Supervisor)**

   Signature... [redacted] **..... Date.....22/11/2019.....**

2. **Dr Jaclyn Mann (Co-Supervisor)**

Format of dissertation

This thesis was presented as a manuscript format, which included submitted journal articles and prepared journal articles under peer review that have emanated from the research project from this field.

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PREFACE AND DECLARATION

This dissertation is submitted in fulfilment of the Doctor of Philosophy degree in Medical Microbiology with the University of KwaZulu Natal, College of Health Sciences. This research has been conducted under supervision of Professor Takafira Mduluza and co-supervision of Dr Jaclyn Mann during the period January 2015 to November 2019.

DECLARATION 1: PLAGIARISM

I **Amos Marume** declare that;

The work presented in this thesis is my original work except where otherwise stated.

This dissertation has never been submitted for any degree or examination at any other institution or university.

This dissertation does not contain any personal information, pictures, graphs or even data except if referenced or acknowledged as outside sources.

No pasted graphics, text, pictures, figures or tables from the internet are found in this dissertation unless specifically acknowledged or properly referenced.

Signed:  **Date: 22 November 2019**

DECLARATION 2: PUBLICATIONS AND MANUSCRIPTS

Part of this work has been presented in the format of a book chapter we published during review of the field of study and manuscripts that are under review in various peer reviewed journals.

Book Chapter

Global Control Efforts of Schistosomiasis and Soil-Transmitted Helminthiasis (Published)

Mduluza T., Chisango T., Nhidza A. F., **Marume A.** (2017). **Global control efforts of schistosomiasis and soil transmitted helminthiasis. In: Luis Rodrigo. Human Helminthiasis**. Croatia. Intech. p 121-148. Available from: http://www.intechopen.com/books/human-helminthiasis/global-control-efforts-of-schistosomiasis-and-soil-transmitted-helminthiasis

Author contributions

TM, TJC, AFN, and AM researched and transcribed different sub–topics within the area of study. TM reviewed and compiled the sub-topics into one chapter. All the authors critically reviewed the chapter before submission, as well as attending to all reviewer comments.

Original research article 1

Marume A, Vengesai A, Mann J, Mduluza T. (2020), **Interleukin-10 and tumour necrosis factor alpha promoter region polymorphisms and susceptibility to urogenital schistosomiasis in young Zimbabwean children living in *Schistosoma haematobium* endemic regions.** *S Afr J Infect Dis.;35(1), a11. https://doi.org/10.4102/sajid.v35i1.11*

Author contributions

AM is the corresponding and principal author. AM, AV and TM developed the field study design, conducted field and sampling work, immunoassays and analysed the data. TM and JM supervised the work. All the authors critically reviewed the manuscript before submission, as well as attending to all reviewer comments.

Manuscript 2

Amos Marume, Theresa Chimponda, Arthur Vengesai, Caroline Mushayi, Jaclyn Mann and Takafira Mduluza, **TNF-α and IL-10 -819 T>C Single Nucleotide Polymorphisms Effects on Urogenital Schistosomiasis in Pre-school Children in Zimbabwe, (Accepted for publication – African Journal of Laboratory Medicine)**

Author contributions

AM is the corresponding and principal author. AM, TC, AV, and TM developed the field study design, conducted field and sampling work, immunoassays and analysed the data. TM and JM supervised the work. CM helped in conceptualization and analysis of the data. All authors contributed to the drafting of the manuscript.

Manuscript 3

Vengesai Arthur, **Marume Amos**, Midzi Herald, Kasambala Maritha, Naicker Thajasvarie and Mduluza Takafira, (2020), **Association of TNF (rs1800629) promoter polymorphism and schistosomiasis with sub-microscopic asymptomatic *Plasmodium falciparum* infections in a schistosomiasis-endemic area in Zimbabwe**. Tropical Medicine & International Health. 26. 10.1111/tmi.13527.

Author contributions

AM is the co-principal author. AV, AM, TC, KH, TC and TM developed the field study design, conducted field and sampling work, immunoassays and analysed the data. TM and JM supervised the work. All authors contributed to the drafting of the manuscript.

Review manuscript

A Marume, J Mann, T Mduluza, **Overview of Schistosomiasis Chemotherapy using Praziquantel (Submitted – African Journal of Primary Health Care & Family Medicine)**

Author contributions

AM researched and transcribed different sub–topics within the area of study and drafted the manuscript. JM and TM reviewed and supervised the work. All the authors critically reviewed the manuscript before submission.

ABSTRACTS AND CONFRENCES

1. **Amos Marume**, (2018), Neglected Tropical Diseases (NTDs): Schistosomiasis and Soil Transmitted Helminths - Abstract, Bryden Bulletin, Bryden Country School
2. **Marume, A.**[*#], Vengesai, A.[#], Mann, J.* & Mduluza, T.*[#] (2018), IL-10 and TNF-α cytokine polymorphism frequencies and susceptibility to *Schistosoma haematobium* in young Zimbabwean children, School Of Laboratory Medicine And Medical Sciences, University of KwaZulu Natal, Durban, South Africa, Research day - Abstract and Poster Presentation.
3. **Marume, A.**[*], Vengesai, A.[#], Midzi, H.[#], Kapungu, N. N.[#],Kwitshana-Mkhize Z.[*]&Mduluza, T.[*] (2016), Impact of single nucleotide polymorphisms on immune responses in *P. falciparum* and S. *haematobium* infections, School Of Laboratory Medicine And Medical Sciences, University of KwaZulu Natal, Durban, South Africa, Research day - Abstract and Poster.

DEDICATION

To my wife, children and late parents.

ACKNOWLEGMENTS

I would like to express my heartfelt gratitude to my supervisors Professor T. Mduluza and Dr Jaclyn Mann for guiding the "ship" throughout. I appreciate assistance received from Arthur Vengesai, Theressa Chiponda and other laboratory mates; as well as from laboratory technicians at the Biochemistry department, University of Zimbabwe and School of Laboratory Medicine, University of KwaZulu Natal. Above all I would like to thank God Almighty for guiding me through my PhD journey.

ABBREVIATIONS

ELISA	–	Enzyme-Linked Immunosorbent Assay
MDA	–	Mass Drug Administration
MRCZ	–	Medical Research Council of Zimbabwe
NTDs	–	Neglected Tropical Diseases
PZQ	–	Praziquantel
SEA	–	Soluble Egg Antigen or Schistosoma Egg Antigen
Sh	–	*Schistosoma haematobium*
SWA	–	Soluble Worm Antigen
TPA	–	Tissue Plasminogen Activator
WHO	–	World Health Organization
χ^2	–	Chi-Square Test

ABSTRACT

Background: Schistosomiasis remains a public health threat in sub-Saharan Africa which carries 85% of the global burden. With effective vaccines a distant future away, and no one all-round intervention, research is still required to ensure that only effective programmes are introduced and implemented as well as evaluated and/or monitored. It is therefore key for researchers, policy makers and implementers to understand the epidemiology, immunology, immunopathology, immunogenetics, chemotherapy, management and control of *Schistosoma haematobium* for optimal elimination strategies to be implemented. A research study was therefore instituted to determine the prevalence, risk factors and host immunogenetic factors in S. haematobium infections among pre- and school going children living in endemic regions of Manicaland and Mashonaland central provinces in rural Zimbabwe. The relationship between single nucleotide polymorphisms (SNPs) of the promoter regions of tumor necrosis factor alpha (TNF-α or rs1800629) and interleukin- 10 (IL-10 or rs1800871) and susceptibility to *Schistosoma haematobium* was investigated. In addition, the relationship between these SNPs and cytokine levels, as well as the relationship between actual cytokine levels and susceptibility to *Schistosoma haematobium* was assessed.

Methods: In this cross-sectional immune-epidemiological study *Schistosoma haematobium* was diagnosed by the microscopic examination of urine specimens for the presence of parasite eggs using the urine filtration technique. DNA for the genotyping was extracted from approximately 300µl whole blood using the QiagenFlexiGene DNA extraction kit, following the manufacturer's protocol. IL-10 and TNF-α promoter region single nucleotide polymorphisms were genotyped using amplification refractory mutation system-polymerase chain reaction (ARMS-PCR).The allele frequencies and genotype distribution of *S. haematobium* infected and uninfected participants were then analysed using the chi-square test. All analyses were performed using SPSS (version 21) and p-values <0.05 were considered statistically significant. The levels of the cytokines (IL-10 and TNF-α) were measured by indirect enzyme linked immunosorbent assay (ELISA) using MABTECH, 3510-1H-6 kits, according to the manufacturer's instruction.

Results: The overall prevalence of *S. haematobium* among children in endemic rural and farming communities of the two provinces of Zimbabwe assessed was 17.1% (158/924). Gender specific prevalences were similar (17.5% in girls and 16.7% in boys; p = 0.735). Age and location were significant risk factors for schistosomiasis in children living in endemic regions surveyed. The older the child the higher the risk of getting infected by *S. haematobium*

(10.5% in 0-5 year olds; 24.0% in 6-10 year olds and 30.7% in 11-15 year olds; $p < 0.001$). IL-10 -1082 G/A, IL-10 -819 C/T and TNF-α -308 G/A single nucleotide polymorphisms were not significantly associated with susceptibility to *S. haematobium* infection. TNF- α genotypes AA, GA and GG were associated with high, moderate to high and low production of TNF-α respectively. IL-10 TT, CT and CC genotypes were associated with low, moderate and high IL-10 plasma levels respectively. Higher TNF-α and lower IL-10 serum levels were negatively associated with schistosomiasis infection. Praziquantel treatment reduced prevalence among the study participants as reinfections were only recorded in 6 of the 59 (10.2%) who were infected at baseline of children.

Discussion and Conclusion: The determined schistosomiasis prevalence puts the regions of Zimbabwe studied within the moderate range as described by the World Health Organisation (10 – 49% prevalence), hence more concerted efforts are required to fight schistosomiasis. Although cytokine genotypes were associated as expected with cytokine levels, genotypes did not directly correlate with schistosomiasis infection while cytokine levels did. This indicates that circulating TNF-α and IL-10 levels are a result of many factors apart from genotypes. Taken together with previous work, this study suggests that high TNF-α and low IL-10 serum levels confer protection against schistosomiasis infection. Since schistosomiasis prevalence was similar between boys and girls and prevalence was high in all age groups (increasing with age), all programmes aimed at eliminating schistosomiasis should include both genders and children of all age groups. Specific locations could be targeted in resource limited settings as location was a significant risk factor for infection. Praziquantel is effective, with few reinfections observed, and therefore remains central in schistosomiasis management. To clearly understand the role host genetic factors in infection and to inform effective control, elimination and eradication programmes, more research on risk factors and host immunogenetics is necessary.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1. Helminths: History and Classification of Schistosomes

The word helminths comes from a Greek word, hélmins, which means a kind of a worm. Helminths have afflicted humans since prehistorical and biblical times and their eggs have been found in mummified bodies dating thousands of years ago (Hotez et al. 2008). They fall into the phyla Nematoda (roundworms) and Platyhelminthes (flatworms) and are very important and notorious human and animal parasites (Brindley et al. 2009). Not all is negative though - for example, history has it that schistosome flatworms saved Formosa, the nowadays Taiwan, from Mao's troops when acute schistosomiasis sickened them long enough for the Americans to arrive (Hotez et al. 2008). Geo-redistribution has left helminths now unduly afflicting populations from marginalized, low-income and resource-constrained portions of the world such as sub-Saharan Africa, Asia and the southern Americas, where at any given time over a billion people are infected by at least one helminth species (WHO 2012).

Diseases caused by helminths form part of the neglected tropical diseases (NTDs) as defined by the World Health Organisation (WHO), and research within the affected communities has shown that parasitic helminths contribute to morbidity, allergic diseases, stunted growth in children, enhanced transmission and progression of HIV/AIDS and malaria, chronic anaemia, unbearable public health and budgetary burdens, poverty, low economic growth, disabilities, and infertility, among other negative consequences (Evans and Stephenson 1995; Crompton et al. 2003; Brindley et al. 2009; WHO 2012; Lwanga et al. 2012; Helmby 2015).

Acute or chronic schistosomiasis (bilharzia) is caused by parasitic dimorphic *Schistosoma* trematode worms, belonging to the phylum Platyhelminthes, which infect humans who come into contact with infested waters as they carry-out their routine agricultural, domestic, occupational and recreational activities (Adenowo et al. 2015; Mupakeleni et al. 2017). Five species, namely *Schistosoma mansoni, Schistosoma haematobium, Schistosoma japonicum, Schistosoma mekongi* and *Schistosoma intercalatum*, are known to infect humans but only *S. mansoni* and *S. haematobium* are of importance to sub-Saharan Africa (Adenowo et al. 2015). *Schistosoma haematobium* causes urinary schistosomiasis, while *S. mansoni* causes intestinal schistosomiasis (Sady et al. 2013).

Schistosomiasis was first described as a human disease in 1851 by a German physician Theodor Bilharz hence the name bilharzia (Chimbari 2012). Bilharz discovered *S. haematobium* during autopsy at Kasr El Ainy hospital, Egypt (Barakat 2013; Jourdan 2013). The description has led to the study of its life cycle. Leiper fully described the schistosome's life cycle in 1915 (Barakat 2013). Endemicity of schistosomiasis in Egypt can be traced back to the Pharaohs' times as shown by the presence of calcified ova/eggs in the Egyptian mummies (Mostafa et al. 1999). Symptoms and signs consistent with schistosomiasis have been found in Egyptian papyrus fragments that date back to 1900BC (Jourdan 2013). Ruffer in 1910 was the first to diagnose *S. haematobium* infections in mummies dating back to the 20th Dynasty (Barakat 2013). ELISA was used to identify *S. haematobium* in two mummies aged over 3000 and 4000 years (Barakat 2013). In Zimbabwe schistosomiasis has persisted since 1909. Estimates of 1961 suggest that as many as 80% of black Zimbabweans were affected (other races were also affected but at much lower rates) (Sokolow 2016).

1.2. Life Cycle of Schistosomes

Understanding clearly the life cycle of schistosomes is of paramount importance if effective management and control policies are to be crafted. The parasite has a complex life cycle bearing well-adapted stages for free-living as well as for life in the intermediate host (snails) and definitive host (vertebrates), where they mature into male or female worms in the host's veins (Walker 2011; Colley et al. 2014). Adult male–female schistosome pairs reside in the mesenteric veins (*S. mansoni*) and lay eggs, which sustain both transmission and pathology (Olliaro et al. 2014). Those schistosome pairs can survive in human veins laying eggs for 3-10 years or even several decades (Colley and Secor 2014).

The eggs secreted via faeces or urine contaminate the environment. When trapped in the definitive host's tissues they cause inflammation and eventually die. Those that get into the environment, if they reach fresh water, hatch into ciliated miracidia which upon infecting a suitable intermediate snail host develop and reproduce asexually to produce thousands of cercariae. Cercariae released into water are ready to infect humans **(FIGURE 1.1)** (Colley and Secor 2014). Cercariae, which penetrate human skin, mature into egg producing females and males in 5-7 weeks. Eggs usually just have a 1-2 week life-span (Colley and Secor 2014). **TABLE 1.1** compares *S. haematobium* and *S. mansoni* in terms of key features in the schistosome's life cycle as hosts.

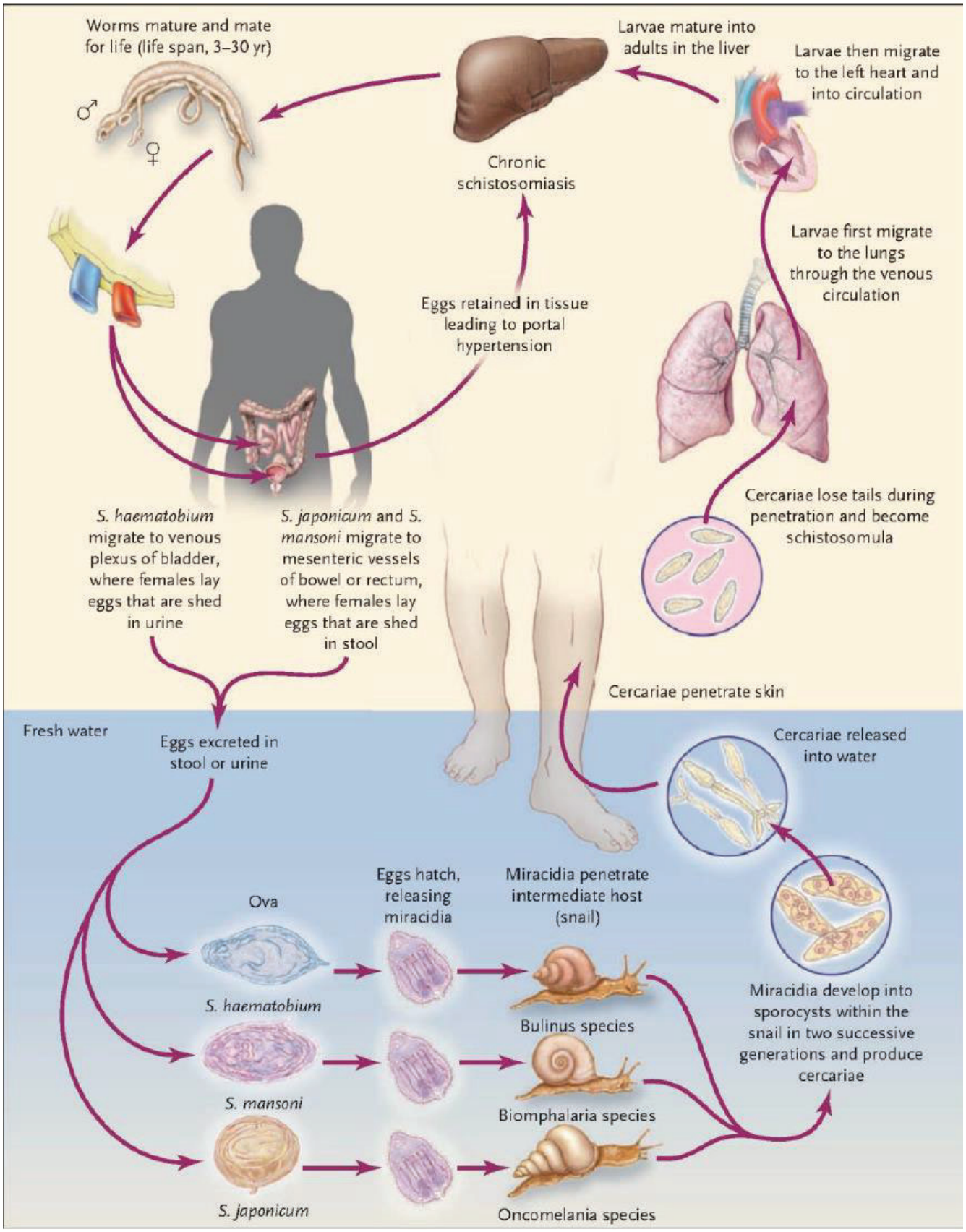

Figure 1.1: Life cycle of schistosomes (López 2015)

Table 1.1: Comparison of key features in schistosome life cycles (Meurs 2014)

Description	***S. haematobium***	***S. mansoni***
Intermediate host snail	 *Bulinus globosus*	 *Biomphalaria pfeifferi*
Definitive host	Human	Human (also primates and rodents)
Location adult worm	Veins of the pelvis	Veins of the mesenteric plexus
Egg excretion via	Urine	Faeces
Egg morphology	 Terminal spine	 Lateral spine
Organ-specific chronic disease	Urinary and genital	Intestinal and hepatic
Geographic distribution	Africa and Middle East	Africa, Middle East, Caribbean and South America

1.3.Clinical Features and Outcome of Schistosomiasis

Symptoms of schistosomiasis include weakness, haematuria and proteinuria (urinary schistosomiasis), diarrhoea, hepatosplenomegaly and carcinoma of the intestine, liver, uterus and bladder **(TABLE 1.2 and TABLE 1.3** show clinical phases and discrete or common symptoms, respectively**)** (Mostafa et al. 1999). Schistosomiasis impairs cognitive development, hence it interferes with the ability of children to achieve much in school and life in general. Blood loss and nutrient absorption impairment may lead to anaemia, especially with higher intensities of infection (Midzi et al. 2014; Friedman et al. 2017). Urinary schistosomiasis has been linked to increased HIV infections due to chronic inflammation and possible ulcerations. Other consequences of urinary schistosomiasis are low birth weights if it affects pregnant women, low worker productivity and poor socio-economic development (Midzi et al.

2014; Friedman et al. 2017). Undernutrition through suppression of appetite and inflammation-mediated cachexia are reported as a consequences of schistosomiasis (Friedman et al. 2017).

Table 1.2: Clinical phases and symptoms of schistosomiasis (Vale et al. 2017)

Phase	**Symptoms**
Immediate	Acute, pruritic and maculopapular eruption at site of cercarial skin penetration within 1 day following exposure
Acute	Systematic hypersensitivity reaction against migrating schistosoma, fever, fatigue, myalgia, malaise, non-productive cough, eosinophilia, patchy infiltrates, weight loss, dyspnoea, diarrhoea, diffuse abdominal pain, toxaemia, hepatosplenomegaly, widespread rash
Chronic	Affects gastrointestinal and urogenital tracts, leading to hepatosplenic and pelvic organ diseases, portal and pulmonary hypertension, abdominal ascites, upper gastrointestinal varices and haemorrhage, female genital schistosomiasis, infertility, increased risk of HIV-1 transmission and squamous cell carcinoma of the bladder

Table 1.3: Discrete and shared effects of *S. haematobium* and *S. mansoni* (Ogden et al. 2013)

S. mansoni **(Hepato-Intestinal Schistosomiasis)**	**Shared effects**	***S. haematobium*** **(Urogenital Schistosomiasis)**
• Abdominal pain • Diarrhoea • Blood in stool • Liver, spleen enlargement in advanced cases • Can result in death	Fatigue and malaise: • Reduced ability to concentrate and learn • Reduced productivity and economic gains	• Blood in urine • Female genital schistosomiasis: lesions of the cervix and vagina, vaginal bleeding and pain during sexual intercourse; a risk factor for sexually transmitted infections • Men: pathology of the seminal vesicles, prostate and other organs, leading to infertility and possible bladder cancer

Schistosomiasis can also be classified as light, moderate and heavy infection based on egg counts per sample (urine or faeces) **(TABLE 1.4)**.

Table 1.4: Classification of infection intensities (Meurs 2014)

Class	***S. haematobium* (eggs per 10ml urine)**	***S. mansoni* (eggs per gram of stool)**
Light	1 – 10	1 – 99
Moderate	11 – 49	100 – 399
Heavy	50 ≤	400 ≤

1.4. Epidemiology and Morbidity of Schistosomiasis

Schistosomiasis is one of the most prevalent NTDs, with schistosomes infecting between 200 to 300 million people worldwide, mostly in poverty stricken regions in the tropics and subtropics **(FIGURE 1.2)** (Sady et al. 2013; Colley and Secor 2014; Midzi et al. 2014; Swartz et al. 2015; Senghor et al. 2015; Sokolow et al. 2017; Mupakeleni et al. 2017). More than 90% of about 790 million people at risk of schistosome infection worldwide are in sub-Saharan Africa (Sady et al. 2013; Sokolow et al. 2017). Together with geo-helminths and excluding malaria, schistosomes account for 40% of the disease burden in the tropics (Adenowo et al. 2015).

The public health importance of schistosomiasis in Zimbabwe cannot be overemphasized as it has been in the top ten of diseases causing hospital visits and/or admissions for decades (Chimbari 2012). Of all the schistosomes, *S. haematobium* is the most common parasite in sub-Saharan Africa causing urinary schistosomiasis or bilharzia (Mutapi et al. 2007). In Zimbabwe *S. haematobium* is more widely distributed in the Matabeleland South province and dam construction has led to many changes to the distribution patterns country wide (Chimbari 2012). In 2012 the prevalences of *S. haematobium* and *S. mansoni* were 20.8% and 9% respectively. In 2014 overall schistosomiasis prevalence was found-out to be 22.7% (*S. haematobium* 18% and *S. mansoni* 7.2%, where some individuals were coinfected). The fall in prevalences can be attributed to various interventions such as mass drug administrations (MDAs), health promotion and/or education and vector control as a result of the renewed interests in the neglected tropical diseases (NTDs) being driven by WHO (Sokolow 2016).

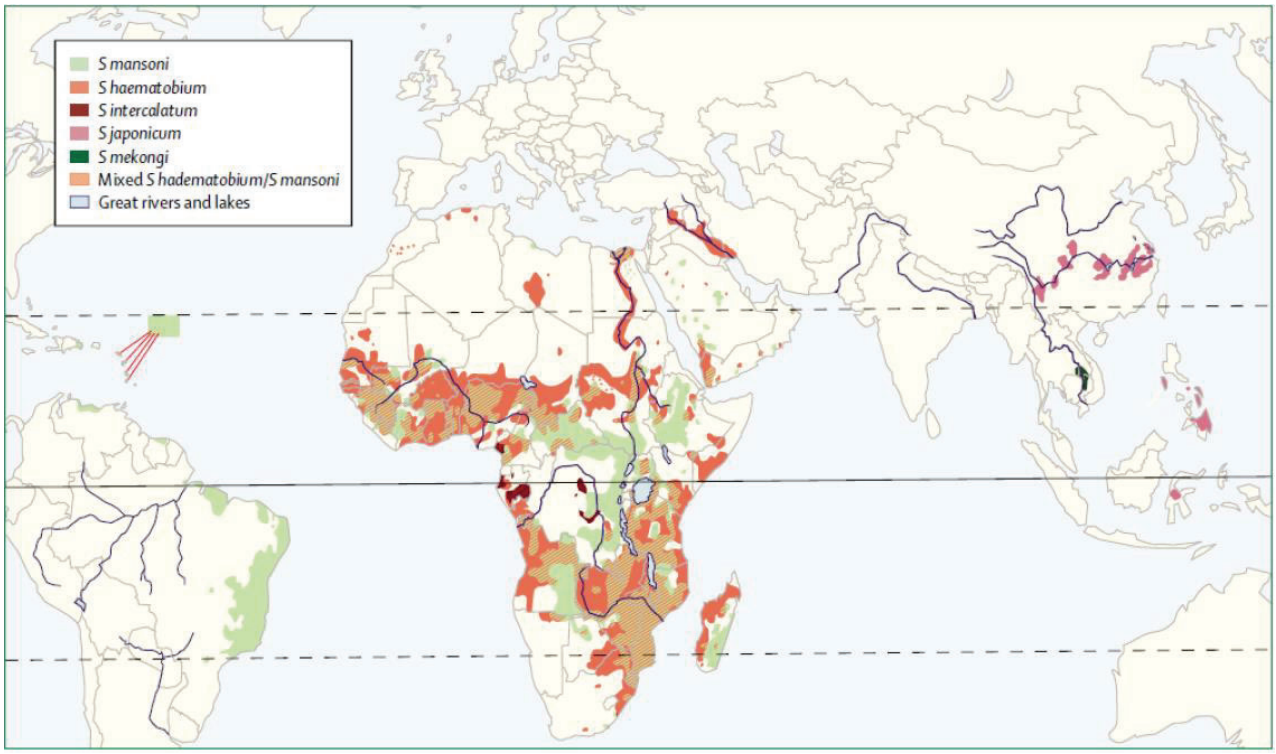

Figure 1.2: Global distribution of Schistosomiasis. From The Lancet Vol. 368, Gryseels et al., 'Human schistosomiasis', pages 1106-18, 2006 as cited by Meurs (Meurs 2014)

Estimates by WHO in 2014 suggests that as many as 40 million women of reproductive age were infected by schistosomiasis (Friedman et al. 2017). In Zimbabwe, in general, schistosomiasis affects boys more than girls and children more than adults due to chores (fetching of water and washing of clothes – **FIGURE 1.3**) and recreational activities/behaviours (swimming) (Sokolow 2016). School-age children are important in the transmission of schistosomes due to unhygienic defecation/urination habits. Many factors have been linked to transmission of schistosomes, namely climatic changes (higher ambient temperatures increased parasitic burden), proximity to water sources, lack of knowledge, age range, man-made ecological changes for example dams and other water reservoirs and socio-economic status (Moyo and Taonameso 2005; Chimbari 2012; Adenowo et al. 2015; Kabuyaya et al. 2017; Mupakeleni et al. 2017).

Figure 1.3: Contact with possibly contaminated water due to chores related activities, exposes children and women to *Schistosoma* (Mduluza and Mutapi 2017)

Coinfections of *S. haematobium* and *S. mansoni* are also common and they lead to within host interactions that may alter disease course. The interactions are based on possible competition for nutrients and mates (infertile pairs) as well as cross-activation of the immune responses (Knowles et al. 2015). Different liver and bladder morbidities often results from such mixed infections (Knowles et al. 2015). As highlighted above, the negative impact attributable to schistosome infection alone includes chronic anaemia, low birth weights, growth stunting, cognitive impairment, infertility, fatigue, low work productivity and increased susceptibility to co-infections, including sexually transmitted infections such as HIV (Midzi et al. 2014; Swartz et al. 2015). Schistosomiasis together with malaria and soil-transmitted helminthic infections is linked to high maternal and child morbidity and mortality, anaemia and malnutrition in sub-Saharan Africa (Sousa-figueiredo et al. 2012). Polyparasitism, leading to synergistic interactions and more detrimental health consequences, is not uncommon as these parasitic infections often overlap geographically (Sousa-figueiredo et al. 2012).

1.5. Diagnosis of Schistosomiasis

Standard diagnosis of schistosomiasis involves the detection of viable eggs in urine (*S. haematobium*), faeces (*S. mansoni* and *S. japonicum*) or tissue biopsies (Colley et al. 2014; Mduluza et al. 2017). Thus prevalence in populations is also estimated using the Kato-Katz

faecal smear (*S. mansoni*) and urine filtration and/or questionnaires for visible haematuria (*S. haematobium*). These traditional ways of diagnosis while efficient in areas of high endemicity are however less sensitive in people with low levels of infection (Nausch et al. 2014). Biomolecular methods, for example the detection of parasite specific DNA in urine (such as PCR *Dra1* tandem repeats detection), if optimized for field use will be essential in schistosomiasis elimination. Antibody based methods can be highly sensitive and specific. These can be easily optimized and put into cheaper rapid diagnosis test (RDT) kits for wider field and point of care (POC) applications especially for resource limited settings. Some useful and commercially available POC tests for schistosomiasis include reagent strips for detection of micro-haematuria (e.g. Hemastix ®, Combur 10 Test® strips and Medi test combi 9) and the POC circulating cathodic antigen (CCA) test (Rapid Medical Diagnostics, Pretoria, South Africa) (Nausch et al. 2014). Each has its advantages and disadvantages for example CCA is sensitive to *S. mansoni* and less so to *S haematobium* and relatively costs more (Nausch et al. 2014).

Other effective techniques include *S. mansoni* cercarial transformation fluid (SmCTF) which detects anti-schistosome antibodies in human blood and schistosome soluble egg antigens enzyme-linked immunosorbent assays (SEA-ELISA). These test though needing more evaluations they have given hope for RDTs in rapid prevalence mapping of *S. mansoni* and *S. haematobium* in schistosome-endemic areas (Nausch et al. 2014). *Schistosoma haematobium* can be diagnosed easily and relatively cheaper using a useful and sensitive ELISA based antibody detection method from urine (Elkawaz and Ghaffarifar 2009).

1.6. Management and Control of Schistosomiasis

There are four basic ways of intervention in the control and/or management of schistosomiasis namely:

1. Treatment of infected individuals which removes and/or reduces morbidity, mortality and environmental contamination levels of schistosome eggs.
2. Proper sanitation that also reduces environmental contamination as well as minimizes the chances of miracidia finding and/or penetrating the schistosome's intermediate hosts i.e. snails.

3. Control of intermediate snail hosts by biological, physical and/or chemical methods e.g. through the use of ducks and prawns thus significantly reducing the levels of cercariae available to infect humans.

4. Provision of and access to safe water minimises chances of cercariae finding the definitive hosts (i.e. humans) in its limited lifespan (Chimbari 2012).

Countries and regions may have different emphasis on each and one of the above mentioned approaches to control schistosomiasis. The WHO emphasises the treatment of infected individuals. Also some approaches are not applicable in some settings because of logistical, financial and/or environmental reasons for example chemical control of snails is not practical in communal areas (Chimbari 2012). Combining two or more strategies often produces better results like what was done in Hippo Valley Sugar Estates, Zimbabwe where chemical and biological control of snails was combined with treatment of the infected individuals. The resultant reduced prevalence persisted for decades after the end of the program (Sokolow 2016).

1.6.1. Intermediate Host Control

Vector control is based on snail killing through use of pesticides or chemicals like niclosamide, habitat changes, predators (for example use of prawns) and biological competitors (Colley et al. 2014; Swartz et al. 2015; Sokolow et al. 2017). *Schistosoma mansoni* and *S. haematobium* parasites are transmitted through their intermediate snail hosts *Biomphalaria pfeifferi* and *Bulinus globosus*, respectively, with both snail species common in most bodies of fresh water (Knowles et al. 2015; Sokolow 2016). Niclosamide can be used to control of intermediate host populations although it is not effective or applicable in communal settings (Chimbari 2012). *Thiara (Tarebia) granifera (Lamarck)* a *Prosobranch* snail can in a time dependent fashion suppress *Biomphalaria* the intermediate host snails for *S. mansoni*(Sodeman 1991). The other competitor snail that was used in Zimbabwe is *Bulinus tropicus* (Sokolow 2016).

Ducks are also useful as biological predators of the intermediate snail hosts of schistosomes. Molluscicides derived from plant sources such as *Phytollacca dodecandra* and *Jatropha curcas* have been shown to be extremely potent in controlling snails although community practicality is still a significant challenge. Prawns and fish (Cichlid species, *Sargochromis codringtonii*) have also been shown to be effective in controlling schistosome's intermediate snail hosts; however the fish does not necessarily prefer the snails and vegetation in the water bodies can

hinder their effectiveness (Sokolow 2016). Furthermore the predator-prey equilibrium may limit effectiveness of biological interventions (Chimbari 2012).

1.6.2. Safe Water and Sanitation

Provision of clean safe water and sanitation helps not only in schistosomiasis control but also in many other NTDs or communicable infections like soil-transmitted helminths, cholera, typhoid and dysentery (Chimbari 2012). By 2015, 76.9% of Zimbabweans had access to improved drinking water and only 36.8% had access to improved sanitation (Sokolow 2016). This further highlights significant work still to be done, especially in the sanitation front, if Zimbabwe is to eliminate schistosomiasis.

1.6.3. Chemotherapy in Schistosomiasis: Praziquantel

Antimony potassium tartrate (APT) is the oldest recorded anti-schistosome drug dating back to 1605 (Trainor-Moss and Mutapi 2016). Orally administered drugs useful nowadays are metrifonate (effective against urinary schistosomiasis and reduces hookworm bio-burdens) and praziquantel (effective against both urinary and intestinal schistosomiasis and other helminths) (Evans and Stephenson 1995; Mduluza et al. 2017). Because praziquantel (PZQ) is relatively safe (even in pregnancy, nursing mothers, children and geriatric patients), effective against all mature/adult *Schistosoma* species, and easily administered at relatively low cost, it is the drug of first choice (Colley et al. 2014). The costs of treating one patient has fallen as from the 1990s from approximately $4 to about $0.30; affording large scale community PZQ administrations (Koukounari et al. 2007). The drug was discovered in Germany by Bayer; was first developed for veterinary use and later for humans (Reich and Govindaraj 1998).

It is estimated that in 2018 as many as 235 million people will be treated using PZQ equating to around 645 million PZQ tablets used (Olliaro et al. 2014). PZQ can be used yearly or regularly in children alone or in conjunction with other drugs managing other parasitic infections, and it can be used through school- or community-based mass drug administration programs, independent of reinfections levels, as preventative and/or morbidity control efforts (Colley et al. 2014). As noted above preventative chemotherapy alone is not enough other strategies mentioned above (vector control, hygiene and sanitation, health promotion and educations) are employed in the effort to eliminate schistosomiasis and other helminthic infections (Midzi et al. 2014).

PZQ is effective given either as a single or divided dose, usually at night after food to minimize side effects and enhance absorption (Castro et al. 2002; Lima et al. 2011; Alsaqabi and Lotfy 2014). It usually comes as a 600mg tablet divided into quarters. The fact that it is taken orally (mimicking normal feeding activities) and as a single dose means compliance is high. It has a bitter taste but is often masked by excipients thus it is supposed to be swallowed whole to avoid the bitterness. These factors makes PZQ well suited for field and/or mass drug administration usage. The accepted dosage world-over, with cure rate ranging between 60-95%, is 40mg/kg body weight (Kallestrup et al. 2006). The drug is manufactured as a racemic mixture of R and S enantiomers (Meister et al. 2016). Chemically PZQ is a synthetic heterocyclic isoquinoline-pyrazine derivative known as 2-(cyclohexylcarbonyl)-1,2,3,6,7, 11b-hexahydro-4H-pyrazino [2, 1-a] isoquinolin-4-one (Masimirembwa et al. 1993; Moore 2012; Alsaqabi and Lotfy 2014) with the molecular formula $C_{19}H_{24}N_2O_2$ and structural formula given in **FIGURE 1.4**.

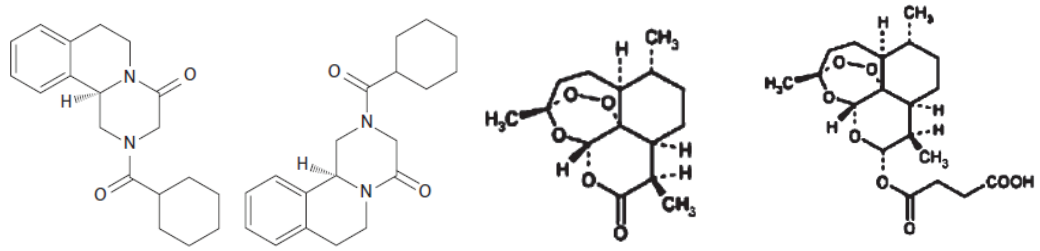

Figure 1.4: Structural formulas of R - praziquantel, S – praziquantel, artemisinin and artesunate respectively (Masimirembwa 2013; Chan et al. 2017; Olliaro et al. 2014)

The enantiomer R-PZQ is believed to be the one with the antischistosomal activity, while the inactive S-PZQ is suspected to be responsible for the unnecessary doubling of the tablet size, bitter taste and the mild to moderate adverse events of PZQ treatment (Reinhard-Rupp and Klohe 2017; Kovač et al. 2017; Olliaro et al. 2014; Meister et al. 2016; Chan et al. 2017; Mduluza and Mutapi 2017). Because it has been demonstrated that enantiomerically pure R-PZQ can be economically produced, it is envisaged that such a formulation will be better acceptable, especially for children (Olliaro et al. 2014; Reinhard-Rupp and Klohe 2017; Mduluza and Mutapi 2017). Some studies have however shown that S-PZQ may have significant activity against *S. haematobium* (Kovač et al. 2017).

1.7. Immunobiology and Pathogenesis of Schistosomiasis

Acute schistosomiasis is rare in people living in endemic areas possibly due to pre- and postnatal exposures. The acute syndrome, also known as Katayama fever, is often accompanied by cercarial dermatitis which also mostly associated with heavy infections in tourists (Boros

1989; Burke et al. 2009). It is characterised by fever, malaise, hepatosplenomegaly, eosinophilia, diarrhoea, and, in some cases, oedema, urticaria, lymphadenopathy and arthralgia (Boros 1989).

Immune responses to schistosome infections can be viewed in many facets, namely immunopathogenesis, resistance to reinfection, and immunodiagnostics (Colley et al. 2014; Mduluza et al. 2017). The first 3-5 weeks of exposure to immature parasites is characterised by type 1 immune response activation leading to the increased Th1 cells and release of IL-12 and interferon (IFN)-γ (Hams et al. 2013). As the parasite matures and start producing eggs (from 5-6 weeks), the immune response shifts to predominantly type 2 kick-starting chronic schistosomiasis. IL10 is thought to be key to the Th1 to Th2 switch (Burke et al. 2009). Chronic schistosomiasis is the most prevalent form of bilharzia in endemic areas as repeated exposures leads to reinfections (Colley et al. 2014). **TABLE 1.5** shows some notable pathology, signs and symptoms of schistosomiasis depending on the infecting schistosome.

Table 1.5: Pathology of human schistosomiasis (Vale et al. 2017)

Species	**Pathology, Signs and Symptoms**
S. haematobium	Urogenital tract fibrosis, female genital schistosomiasis, bladder cancer, renal failure, infertility
S. mansoni	Liver/periportal fibrosis, hepatomegaly, intestinal fibrosis, diarrhoea

The chronic disease is due to immunopathological reactions against trapped schistosome eggs in organs like the liver, intestines, bladder and the urogenital system (Madinga et al. 2015). This is because the schistosome eggs release highly antigenic glycoproteins collectively known as schistosoma egg antigen (SEA) through microscopic pores within the rigid egg shell, which then promote dominant Th2 immune responses (Boros 1989; Joshi et al. 2008). Schistosome eggs can be deposited in the intestinal tract, and liver, renal, pulmonary or central nervous systems leading to the granulomatous response (Boros 1989). As stated above, the immune responses are driven by Th2 through recruitment and activation of eosinophils, alternatively activated macrophages (or M2 macrophages), dendritic cells and $CD4^+$ Th2 cells (Joshi et al. 2008). The egg-antigen also leads to the production of IL-4, IL-5, IL-13 and immunoglobulin

(Ig) E (Hams et al. 2013). **FIGURE 1.5** summarizes the components and cytokine contributions to granulomatous response.

The type 2 immune response peaks with marked granulomatous inflammation around schistosome eggs. With chronic infection a state of hyporesponsiveness ensues characterised by dampening of Th2 responses leading to controlled chronic disease. This is in part as a result of a dynamic association between Th1, Th17 and T regulatory cells which regulates disease severity (Hams et al. 2013). Pro-inflammatory Th17 $CD4^+$ T cells, which are implicated in other autoimmune disease like encephalomyelitis and collagen-induced arthritis, are also implicated in the granulomatous response. Fibrosis that follows the granulomatous inflammatory responses enhances the disease pathology contributing to mortality (Boros 1989; Joshi et al. 2008). Though this immune response is key for host survival, the resultant chronic granulomatous response is deleterious to any tissue containing those eggs. The granuloma, full of Th2 cells, eosinophils and M2 macrophages, acts to protect the surrounding host tissue from the toxins released by the egg, through providing a physical barrier between the egg and the tissue and sequestration of antigenic products secreted by the egg (Hams et al. 2013). Th2 cytokines, including IL-4 and IL-13, promote immunopathology while IFN-γ protects against the development of severe fibrosis. Thus tight regulation of the Th1, Th2 and Th17 cytokine responses generated during schistosome infection is essential to prevent excessive pathology (Burke et al. 2009).

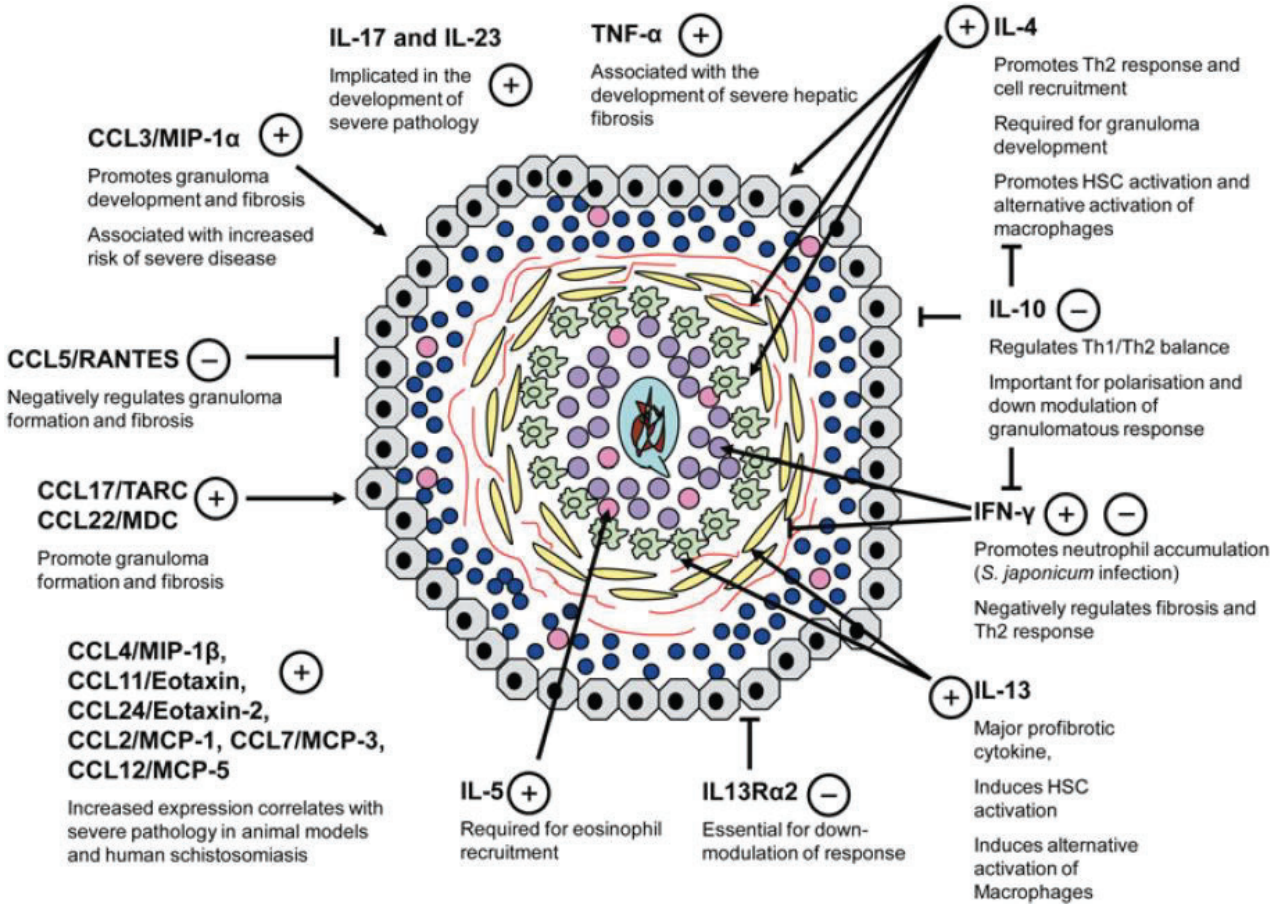

Figure 1.5: Major components of, main cytokines and chemokines that regulate the granulomatous response to schistosome eggs in the host liver (Burke et al. 2009).

The development of protective immunity against schistosome is rather slow and the reasons behind such slowness are not yet clear. Some scientists postulate that dying parasites, which will be scarce given the longer lifespans of the worms, are the source of the protective antigens while others believe that the threshold for exposure to the necessary antigens for protective immunity to develop is rarely reached (Mitchell et al. 2012). Schistosomiasis immunity depends in part on intensity of infection- the higher the intensity of infection, the higher the chances of becoming resistant to schistosomes. Children often have higher burdens and are more likely to be reinfected than adults (Mduluza et al. 2001).

Many scientists have linked some antibodies to the said protective immunity. Several studies in infected individuals have shown heterogeneity in terms of parasitic burdens and types or levels of anti-schistosome specific antibodies (such as IgG2a, IgM, IgG1 and IgG2b) (Mitchell et al. 2012), thereby linking antibodies to protective immunity. Studies in schistosomiasis endemic regions have linked few loci (SM1 of the 5q31-q33) to susceptibility to infection, reinfection, diseases and severity (Mangano and Modiano 2014). Those immuno-epidemiology

studies have also helped emphasise the importance of the host Th2-type immune response in susceptibility to schistosomiasis and other helminthic infections (Mangano and Modiano 2014). Resistance to reinfection has consistently been associated with high levels of IgE antibodies against worm antigens, low concentrations of IgG4 antibodies to worm antigens and high levels blood eosinophilia (Colley et al. 2014). Treatment has been shown to help the development and maintenance of resistance to infection.

1.8. Cytokine Polymorphisms and Schistosomiasis

Different cytokine genotypes that may predispose individuals to severe inflammatory disease with worse clinical outcome exist in the populations, primarily due to the selective pressure of infectious diseases (Kube et al. 2003). Thus individual profiles for immuno-regulatory cytokines like IL-10, IFN-γ and TNF-α is key in determining outcomes of certain diseases (Kube et al. 2003). Polymorphisms in the genes encoding cytokine receptors have also been shown to correlate with increased susceptibility to infection or inflammatory conditions (Erikstrup et al. 2007; Brooks et al. 2008; Naicker et al. 2009; Shrestha et al. 2010). Furthermore, polymorphisms in the cytokine promoters greatly influence the levels of expression of the affected cytokine genes, are linked to certain autoimmune conditions and determine susceptibility to infections (Abel and Dessein 1997; Leornard 2000; Chatterjee et al. 2009).

Schistosomiasis disease severity has also been shown to be influenced by host genetic factors such as cytokine genotypes among other factors including coinfections, infection intensity and duration as well as nutritional status (Mduluza et al. 2017). According to Hirayama (2005), higher frequencies of the HLADRB5* 0101 (B1*1501) allele (HLA class II gene) and IL-13 promoter A/A (IL-13P A/A of IL-13 gene) genotypes are associated with severe fibrosis in chronic schistosomiasis (Hirayama 2005).

The severity of schistosomiasis has also been shown to be influenced by cytokines/interleukins which regulate the inflammatory granuloma formation for example IL10, IL12, TNF-α and IFN-γ. Fibrosis that usually then follows was also shown to be under the influence of IL4, TGFβ1 and IFN-γ (Dessein et al. 1999). Other authors have also reported that TGF-β, IL1, IL13 and IL4 are fibrogenic and IL10 has regulatory role of controlling excessive Th1 and Th2 polarization of the granulomatous response. IFN-γ and IL12 are protective against fibrosis while TNFα is thought to be either protective or proinflammatory (Henri et al. 2002). IFN-γ production by egg-antigen stimulated PBMCs correlates with protection against severe

schistosomiasis, and individuals with severe disease often have low production of the cytokine. IFN-γ is often associated with protection against peripheral fibrosis in humans while IL-10 protects against severe hepatic central fibrosis (Burke et al. 2009).

A Brazilian study provided evidence on strong gene influence on schistosome infection levels. The genes involved were mapped and shown to be in the 5q31-q33 chromosomal regions responsible for Th1/Th2 response regulation (Mduluza et al. 2017). The region has genes for IL4 (Th2 response promoter as well as cellular recruitment), IL5 (key in eosinophil recruitment) and IL13 (a profibrotic cytokine) (Burke et al. 2009). Two single nucleotide polymorphisms within the IL5 gene have been associated with development of schistosome symptomatic infection in Chinese population. Genes in the 6q22-q23 regions were shown to have influence in the hepatic fibrotic responses in *S. mansoni* chronic infection (Burke et al. 2009). The region is associated with the IFN-γ receptor 1 gene encoding for the receptor of the antifibrogenic cytokine IFN-γ. Evidence exists for further association of schistosome infection levels, morbidity and/or mortality with IFN-γ receptor 1 subunit, major histocompatibility complex and other cytokine gene polymorphisms (Mduluza et al. 2017). Studies in Egypt also confirmed linkages of severe schistosomiasis with polymorphisms in the IFNGR1 locus and IL13/IL14 region as well as TGF-β1 gene (Burke et al. 2009). A study done in Brazilian patients failed to find association between IL-10 gene promoter region (G1082A/-C819T/-C592A) polymorphisms and periportal fibrosis regression after specific treatment for schistosomiasis (Silva et al. 2016).

A study done in Kenya confirmed the association between high production levels of TNF-α with exaggerated granulomatous response in the bladder wall/urinary tract pathology in children infected with *S. haematobium* (King et al. 2001). Other studies have also confirmed the aggravating effects of TNF-α and the protectiveness of IFN-γ in periportal fibrosis associated with chronic *S. mansoni* infection (Henri et al. 2002). Collectively, these studies show that IL-10 and TNF-α among other cytokines play a major role in the pathogenesis and severity of schistosomiasis, and that host immunogenetics is paramount in determining susceptibility or resistance to schistosomiasis. In addition, researchers in Kenya have shown that non-synonymous polymorphism in IL23R gene (rs1884444) renders protection against schistosomiasis-associated Immune Reconstitution Inflammatory Syndrome in patients receiving antiretroviral therapy (Ogola et al. 2014), indicating that host immunogenetics as also influences outcomes of drug therapies.

1.8.1. Interleukin-10

Interleukin (IL)-10 is an important anti-inflammatory T helper 2-type and T-regulatory type cytokine produced primarily by monocytes and lymphocytes (Imai *et al.*, 2011; Mutapi *et al.*, 2011). Generally major sources of IL-10 varies from tissue to tissue or during acute or chronic stages of schistosomiasis. T helper 2 responses provide host protection against extracellular parasites such as *S. haematobium* but insufficient or little protection against the majority of the intracellular pathogens (Colley and Secor, 2014; Odegaard and Hsieh, 2014). T helper 2 responses also tend to inhibit harmful T helper 1 inflammatory responses; as part of the down regulatory mechanism against exaggerated inflammatory responses (Odegaard and Hsieh, 2014).

IL-10 limits tissue damage (immunopathology) but interferes with pathogen clearance (Odegaard and Hsieh, 2014). Low IL-10 signalling may result in severe, often fatal immunopathology in schistosomiasis. Excessive production of IL-10 can also inhibit the T helper 1 immune response thus enhance immune evading by pathogens leading to conditions such severe anaemia associated with schistosomiasis. IL-10 is associated with high production IgG4 antibodies that block parasite specific IgE antibodies and susceptibility to schistosome infection; blocking IL-10 receptors has been shown to help in schistosomiasis treatment and protection against reinfection (Wilson *et al.*, 2011; Colley and Secor, 2014).

The human IL-10 gene is approximately 4.7 kb long on chromosome 1q21–32 and contains five exons that are separated by four introns (Mosser and Zhang, 2008). Five SNPs namely; -3575, -2763, -1082, -819 and -592 tagging the promoter region of the gene have been widely studied (Mosser and Zhang, 2008). The homozygous wild type, GG, CC and CC of IL-10 promoter positions -1082, -819 and -592 respectively, has been reported to be associated with high IL-10 production and protection against severe pathology (Guzowski *et al.*, 2005; Zhang *et al.*, 2012). The heterozygous condition and homozygous mutants (AA, TT and AA) associated with moderate and low IL-10 production respectively (Guzowski *et al.*, 2005; Zhang *et al.*, 2012).

1.8.2. Tumour Necrosis Factor alpha

Tumour Necrosis Factor alpha (TNF-α) is a 17 kDa potent immunomediator and proinflammatory cytokine produced by macrophages, monocytes, neutrophils, T cells and NK-cells (Elahi *et al.*, 2009; Tahan *et al*., 2016). TNF-α is reported to be important in apoptosis, migration of white blood cells to sites of infection, activation phagocytes and many other

important cellular processes such as proliferation, differentiation, growth and the immune response in general (Elahi *et al.*, 2009; Tahan *et al*., 2016). Higher levels of TNF-*α* are associated with severe immunopathological conditions or complications as well as potent anti-parasitic activity and rapid clearance of parasites such as schistosomes (Kubota *et al.*, 1998; King *et al.*, 2001).

The human TNF-α gene is located on the short arm of chromosome 6 in a region of the major histocompatibility complex between the class 1 HLA-B and the class 2 HLA-DR loci (Kubota *et al.*, 1998; Van Der Linden *et al.*, 2001). Its full-length spans 2.76-kb and has four exons and three introns (Tu *et al.*, 2018). Genetic polymorphisms in the TNF-α locus are known to be related to several autoimmune, infectious such as schistosomiasis and neoplastic diseases (Kubota *et al.*, 1998). Several TNF-α SNPs that affect TNF-α gene expression levels such as -1031 (T/C), -863 (C/A), -857 (C/A), -851 (C/T), -419 (G/C), -376 (G/A), -308 (G/A), -238 (G/A), -162 (G/A) and -49 (G/A) have been reported (Elahi *et al.*, 2009; Tahan *et al*., 2016; Tu *et al.*, 2018). The -308 SNP directly affect the production of TNF-α. The mutant allele of TNF-α -308A is associated with high levels of TNF-α; high parasite clearance and severe inflammatory conditions (Kubota *et al.*, 1998; Tu *et al.*, 2018).

1.9. Study Rationale and Justification

The lack of any one effective treatment, control and/or management option means there is a need for concerted efforts aimed at eradication of schistosomiasis. Drug resistance and adverse effects of current medicines used to treat and/or prevent schistosomiasis further stress the dire need for better options. Given the disease burden patterns, there is need for sub-Saharan scientists to participate in the ongoing search for alternative drugs, immunopathology, immunoepidemiology and/or anti-schistosomal vaccines.

The current study is linking the presence of cytokine polymorphisms to susceptibility and severity of schistosomiasis as well as to drug outcomes. As highlighted above, other studies have linked IL10 to controlling of excessive Th1 and Th2 polarization in granulomatous response, and TNFα to both protectiveness and proinflammatory responses. Thus polymorphisms on promoter regions of these genes could have strong influence on severity and resistance to morbidity of schistosomiasis of children living in endemic areas of Zimbabwe. This study helps in further understanding host-parasitic interactions in schistosomiasis and helminthiasis in general, protective immunity development and the impact of an individual's immunological profile on disease burden, progression and outcome. This information is

essential in informing prevention, immunoepidemiology research and other schistosomiasis control strategies.

The study contributes valuable information on schistosomiasis epidemiology in rural and farming communities of Zimbabwe as well as highlight key risk factors (mainly host-genetic factors) among children (0-15years). The contributions includes information that will help in the optimization of drug therapies. Thus, in summary, this immunogenetic and immuno-epidemiological study helps with the unravelling of the immuno-epidemiological basis of schistosomiasis susceptibility and/or infection as well as other parasitic infections. Apart from increasing our understanding on immunogenetics and immuno-epidemiology, this study has a positive impact on our parasitic infection treatment, control and prevention strategies as it will help in determining prevalence, groups at risk, risk factors (including host genetic factors) and effectiveness of previous and present interventions.

1.10. Study aims and objectives

1.10.1. Aim

The main aim of this study is to evaluate frequencies of genetic polymorphism in cytokines IL-10 and TNF-α gene promoter regions and susceptibility to *S. haematobium* infections.

Objective

To;

1. Evaluate frequencies of genetic polymorphisms in IL-10 and TNF-α gene promoter regions in both *S. haematobium* infected and unifected children.
2. Determine genetic predisposition and overall susceptibility to urogenital schistosomiasis in children living in endemic areas of Zimbabwe.
3. Investigate impact of age, gender and location on the prevalence of urogenital schistosomiasis in endemic rural and farming communities of Zimbabwe.
4. Make inferences on the potential impact of single nucleotide polymorphisms of TNF-α (rs1800629) and IL-10 (rs1800871) on immunological profiles and drug administration outcomes.

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Southern African Journal of Infectious Diseases
ISSN: (Online) 2313-1810, (Print) 2312-0053

Interleukin-10 and tumour necrosis factor alpha promoter region polymorphisms and susceptibility to urogenital schistosomiasis in young Zimbabwean children living in *Schistosoma haematobium* endemic regions

Authors:
Amos Marume[1,2]
Arthur Vengesai[1,3]
Jaclyn Mann[1]
Takafira Mduluza[1,3]

Affiliations:
[1]Department of Infection Prevention and Control, School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

[2]Paraclinical Department, Faculty of Veterinary Sciences, University of Zimbabwe, Harare, Zimbabwe

[3]Department of Biochemistry, University of Zimbabwe, Harare, Zimbabwe

Corresponding author:
Amos Marume,
stafirejee@gmail.com

Dates:
Received: 08 May 2019
Accepted: 23 June 2020
Published: 03 Sept. 2020

How to cite this article:
Marume A, Vengesai A, Mann J, Mduluza T. Interleukin-10 and tumour necrosis factor alpha promoter region polymorphisms and susceptibility to urogenital schistosomiasis in young Zimbabwean children living in *Schistosoma haematobium* endemic regions. S Afr J Infect Dis. 2020;35(1), a11. https://doi.org/10.4102/sajid.v35i1.11

Background: Host genetic factors can influence susceptibility, morbidity and mortality from schistosomiasis. The study explored the association between single nucleotide polymorphisms (SNPs) in interleukin-10 (IL-10) and tumour necrosis factor alpha (TNF-α) promoter regions and susceptibility to *Schistosoma haematobium* infection.

Methods: Urine specimens were collected from 361 primary school children aged 5–15 years from schistosomiasis endemic areas of Manicaland and Mashonaland central provinces. *Schistosoma haematobium* was diagnosed using the urine filtration method. Only 272 participants provided adequate blood for genotyping. Genotyping was performed using the amplification refractory mutation system-polymerase chain reaction. The association between IL-10 and TNF-α SNPs and *S. haematobium* infection was analysed using the chi-square test.

Results: *Schistosoma haematobium* infection was confirmed in 26.8% of the participants. No significant difference in *S. haematobium* prevalence between men (51.6% of those infected) and women (48.4%) ($\chi^2 = 0.008$, $df = 1$, $p = 0.928$) was observed. The total IL-10 -1082 G, IL-10 -819 C and TNF-α -308G allele distribution between *S. haematobium* infected and uninfected participants was 50.7% and 51.5% ($\chi^2 = 0.025$, $df = 1$, $p = 0.87$), 54.3% and 60.6% ($\chi^2 = 1.187$, $df = 1$, $p = 0.187$) and 82.1% and 80.9% ($\chi^2 = 0.099$, $df = 1$, $p = 0.753$), respectively, and the differences were not significant.

Conclusion: Interleukin-10 -1082 G/A, IL-10 -819 C/T and TNF-α -308 G/A SNPs were not significantly associated with susceptibility to *S. haematobium* infection. The prevalence of schistosomiasis is still in the moderate range and is similar in boys and girls.

Keywords: cytokine polymorphisms; associations; *S. haematobium*; IL-10; TNF-α.

Introduction

Children in sub-Saharan Africa have endured many public health threats that significantly alter their prospects, health and socio-economic development. One of these main health threats, as listed by the World Health Organization (WHO), is schistosomiasis, which is caused by *Schistosoma haematobium* (urogenital) or *Schistosoma mansoni* (intestinal). These helminthic infections affect more than 200 million people worldwide and 800 million people are at risk in 76 endemic countries, leading to annual losses ranging from 1.7 to 4.5 million disability-adjusted life years.[1,2] Some countries have made significant strides in controlling schistosomiasis (Morocco and some Caribbean countries including Sint Maarten, Saint Kitts and Vieques) and a few others have eliminated it (Japan and Tunisia).[3] Schistosomiasis is ranked ninth as one of the most reported outpatient illnesses in Zimbabwe. The overall prevalence of *S. haematobium* and *S. mansoni* in Zimbabwe is 20.8% and 9%, respectively.[4] Risk factors include the number of water bodies, location (rural and agricultural lands have high transmission), poverty, ignorance, age, gender, poor housing, and poor hygiene and sanitation.[5] The disease has been linked to growth retardation, fatigue, weakness, impairment of memory and cognitive reasoning and increased risk of anaemia, leading to poor academic

performance and thus limiting the potential of infected children.[3] Irreversible damages and deaths because of kidney and/or liver damage have been reported especially in elderly populations.[1,2] The disease has been linked to increased chances of acquiring human immunodeficiency virus (HIV); the negative consequences of schistosomiasis are even more pronounced when occurring together with other infections or diseases, for example, HIV infection, cancer and malaria.[3,6] Chemotherapy with praziquantel (PZQ) is highly effective but re-infections are common.[1,2] Furthermore, most people infected with schistosomiasis are asymptomatic, contributing to difficulty in controlling the disease.[3,5]

Host genetic factors, such as genetic polymorphisms altering expression of cytokines key in the Th1/Th2 differential responses, have been recognised as key influencers in parasitic infections, prognosis, morbidity, treatment outcomes and vaccine development.[7,8] Severity of symptoms has been associated with cytokines that influence the granulomatous response, namely, interleukin-10 (IL-10) and tumour necrosis factor alpha (TNF-α). Specifically, lower IL-10 and elevated TNF-α levels are associated with an exaggerated granulomatous response to ova trapped in the bladder wall as well as other urinary tract pathologies.[9] Other cytokines such as IL-4 and TGF-β have been associated with fibrosis after the granulomatous reactions, whilst IFN-γ has been recognised as having anti-fibrogenic effects.[10] Interleukin-10 released by Th2 cells is recognised as an important anti-inflammatory and anti-fibrotic cytokine.[11] Interleukin-10 elevation in the early phase of *S. haematobium* infection is linked to down-modulation of immunopathological responses and hence reduced morbidity;[12,13] however, elevated parasite-specific IL-10 is a risk factor of re-infection.[14,15] Low IL-6, IL-10 and TNF-α and high IL-13 levels have been linked to enhanced *S. mansoni* disease progression.[16] However, other studies have shown that some cytokine polymorphisms, even those known to cause elevated production of TNF-α (namely at -376 and -308), have no link with major developments of hepatic periportal fibrosis (PPF) in *S. mansoni* and *Schistosoma japonicum* infections.[17] Cytokines also influence serum Immunoglobulin E (IgE) levels, which are associated with resistance and/or susceptibility to schistosomiasis in humans.[18] For example, immunity or resistance to schistosome infection has been associated with high and low levels of IgE and Immunoglobulin G4 (IgG4), respectively, and IL-10 alters the production of these antibodies. Interleukin-10 indirectly downregulates IL-4-induced production of IgE and directly upregulates IL-4-induced production of IgG4.[19] Collectively, these studies have shown that IL10 and TNFα amongst other cytokines play a major role in the pathogenesis and severity of schistosomiasis, and that host immunogenetics is paramount in determining the susceptibility and/or resistance to schistosomiasis.

Given the importance of IL-10 and TNF-α in schistosomiasis, this cross-sectional study was designed to determine possible links between single nucleotide polymorphisms (SNPs) in promoter regions of *IL-10* and *TNF-α* genes and susceptibility to schistosomiasis. Given the resources, two interleukins were chosen as they could adequately represent both extremes: the pro-inflammatory (TNF-α) and the anti-inflammatory (IL-10). Interleukin-10 -1082 G/A, IL-10 -819 C/T and TNF-α -308 G/A were chosen because they were associated with other infectious and non-infectious diseases.[18,20] To this end, we performed genotyping of cytokines IL-10 and TNF-α as well as confirmed the presence or absence of *S. haematobium* infection, the most prevalent schistosome infection, in 361 children in endemic areas of Zimbabwe.

Materials and methods

Study population and sampling

The study population was previously described by Midzi et al.[21] Primary school children aged between 6 and 15 years were targeted and recruited for the study as they constitute the high-risk age group for schistosomiasis.[21] Briefly, Manicaland and Mashonaland central provinces in rural Zimbabwe were selected for this study based on their geographical locations (characterised by high annual rainfall and open water bodies, conditions conducive for *Schistosoma* species leading to high schistosomiasis endemicity) and relatively higher prevalences in previous studies.[21] Simple random sampling method was used to select schools per province, Bandanyenje primary school in Manicaland and Bemberi primary school in Mashonaland, using the lottery method. The sample size of 361 for parasitology and 272 for genotyping (some were not willing or able to provide adequate blood sample for genotyping) was calculated using the EPI Info 6 statistical package as previously described by Midzi et al.[21] Briefly, based on the national primary school enrolment of more than 2 million children, the national sample size calculated by Epi Info version 6 was 15 818 as of 2014.[21] The calculated sample size per school was 50 children. The researchers targeted 100 children to cater for errors and the fact that some children were unwilling and/or unable to provide enough blood sample for genotyping. The sample size was higher than what was anticipated because of overwhelming responses.

TABLE 1: Wild-type, mutant primer and generic primer sequences for the determination of human interleukin-10 -1082, interleukin-10 -819 C/T and tumour necrosis factor-alpha -308 G/A promoter region polymorphisms.

Cytokine	Position	Mutation	Primer
IL-10	-1082	A→G	Wild type: TAAGGCTTCTTTGGGAG Mutant: TAAGGCTTCTTTGGGAA Generic: TAAATATCCTCAAAGTTCC
IL-10	-819	C→T	Wild type: CCCTTGTACAGGTGATGTAAC Mutant: CCCTTGTACAGGTGATGTAAT Generic: AGGATGTGTTCCAGGCTCCT
TNF-α	-308	G→A	Wild type: AGGTTTTGAGGGGCATTG Mutant: AGGTTTTGAGGGGCATGA Generic: CAGCGCAAAACTTCCTTGGT

IL-10, interleukin-10; TNF-α, tumour necrosis factor alpha.

Cytokine genotyping

Deoxyribonucleic acid (DNA) for the genotyping was extracted from approximately 300 µL of whole blood using the Qiagen FlexiGene DNA extraction kit, following the manufacturer's protocol. Interleukin-10 and TNF-α promoter region SNPs were genotyped using amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). Two different primers (Inqaba Biotechnology, Pretoria, South Africa; Table 1), specific for wild-type genotype (1 µL) and mutant genotype (1 µL), respectively, were separately mixed with 1 µL (10 mM) generic primer, 0.5 µL 10 mM forward and 0.5 µL 10 mM reverse internal control primers (human growth hormone), 12.5 µL quick load Taq 2x master mix (New England Biolabs, Ipswich, MA) and 4.5 µL of sterile, nuclease-free water (New England Biolabs, Ipswich, MA). About 5 µL of the template DNA was added to the mastermix prior to loading onto a thermocycler (PXE 0.2 thermocycler, Thermo Electron Corporation, Waltham, MA). Interleukin-10 -1082 G/A and IL-10 -819C/T alleles were amplified using the following conditions: 1 min denaturation step at 95 °C; 10 cycles of 15 s at 95 °C, 50 s at 65 °C and 40 s at 72 °C; 20 cycles of 20 s at 95 °C, 50 s at 59 °C and 30 s at 72 °C, followed by cooling at 4 °C.

Amplification refractory mutation system-polymerase chain reaction amplicons were analysed on a 2% agarose gel to score the presence or absence of the cytokine gene polymorphisms (Figures 1–3).

Detection of *Schistosoma haematobium*

Schistosoma haematobium was diagnosed by the microscopic examination of urine specimens for the presence of parasite eggs using the urine filtration technique. In brief, urine specimens were collected from willing participants and 10 mL of urine was filtered through a nitrile filter membrane. The membrane was stained with iodine and examined using a light microscope. Participants who were *S. haematobium* positive were treated with a single dose of PZQ (40 mg/kg of body weight). Bread and orange juice were given as supplementary food to enhance the absorption and nauseating effects of PZQ.

Statistical analysis

The allele frequencies and genotype distribution of *S. haematobium*-infected and uninfected participants were then analysed using the chi-square test. All analyses were

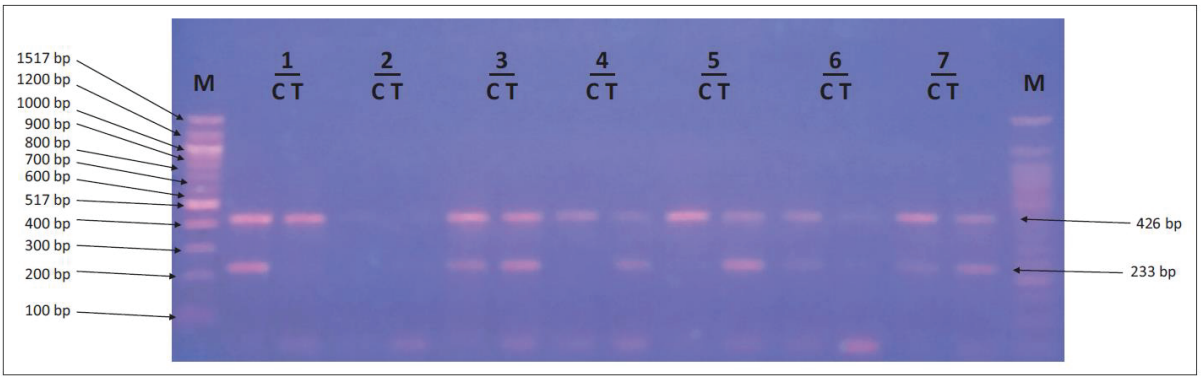

FIGURE 1: Amplicons for interleukin-10 -819 C/T single nucleotide polymorphism. Lane M shows 100 base pair (bp) molecular marker. The 426 bp fragments correspond to the internal control (the human growth hormone gene). The 233 bp fragments were specific for C and T alleles of IL-10 -819 C/T single nucleotide polymorphisms. Lanes labelled 3, 6 and 7 show heterozygous CT genotypes. Lanes labelled 1 and 4 show homozygous CC and TT genotypes, respectively.

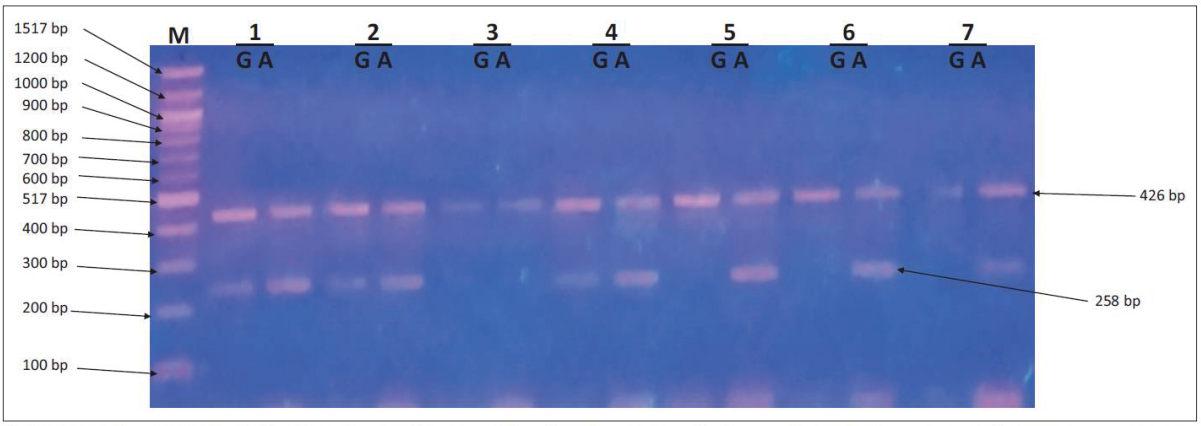

FIGURE 2: Amplicons for interleukin-10 -1082 G/A single nucleotide polymorphism. Lane M shows 100 base pair (bp) molecular marker. The 426 bp fragments correspond to the internal control. The 258 bp fragments were specific for G and A alleles of interleukin-10 -1082 G/A single nucleotide polymorphisms. Lanes labelled 1, 2 and 4 show heterozygous GA genotype. Lanes labelled 5, 6 and 7 show homozygous AA genotype.

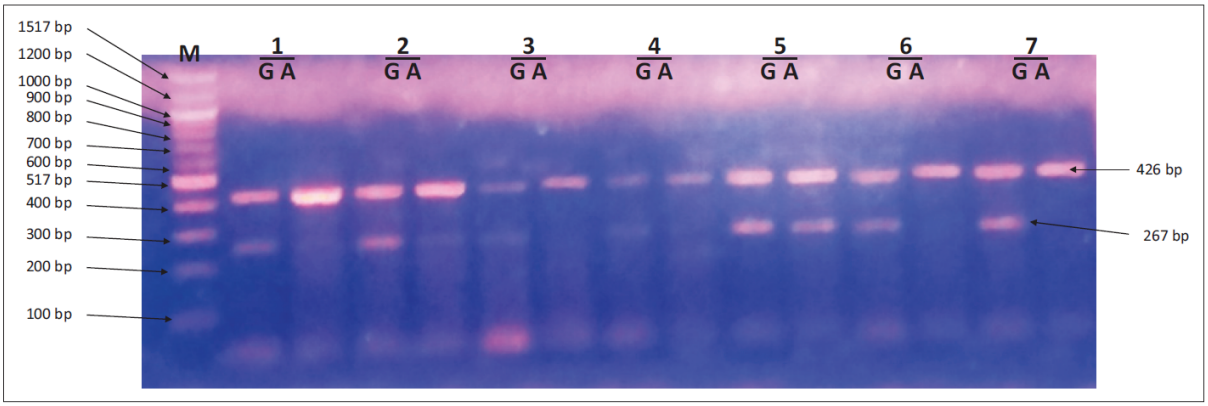

FIGURE 3: Amplicons for tumour necrosis factor-alpha -308 G/A single nucleotide polymorphism. Lane M shows 100 base pair (bp) molecular marker. The 426 bp fragments correspond to the internal control. The 267 bp fragments were specific for G and A alleles of tumour necrosis factor-alpha -308 G/A single nucleotide polymorphisms. Lane 5 shows heterozygous GA genotype. Lanes labelled 6 and 7 show homozygous GG genotype.

performed using Statistical Package for the Social Sciences (SPSS) version 21 and *p*-values <0.05 were considered statistically significant.

Ethical consideration

Blood and urine specimens were obtained from the study participants following the signing of informed consent forms by their parents or guardians to allow their participation. The study was registered and ethically approved by the Zimbabwe's ethics board for biomedical research (the Medical Research Council of Zimbabwe – MRCZ/A/1710). In addition, community leaders, the Provincial Medical Director, the District Medical Officer and education directors also granted permission to conduct the study at Bemberi and Bandanyenje primary schools. The children and parents were informed about the aims, risks and benefits of the study. Participation was voluntary and participants were free to withdraw from the study at any time.

Results

The demographics of the study participants are summarised in Table 2.

Schistosoma haematobium prevalence

Amongst the 361 study participants from Bemberi and Bandanyenje, overall 26.8% (28.9% and 25.8%, respectively) were found to be infected with *S. haematobium*. No significant difference was observed between the prevalence of men infected with *S. haematobium* (51.6%) and that of women infected with *S. haematobium* (48.4%, χ^2 = 0.008, *df* = 1, *p* = 0.928).

Distribution of the interleukin-10 -1082, interleukin-10 819 and tumour necrosis factor-α -308 genotypes and alleles frequencies in uninfected and infected *Schistosoma haematobium* groups in Bemberi

Total genotype and allele frequencies of IL-10 -1082 G/A, IL-10 -819 C/T and TNF-α -308 G/A in Bemberi are shown in Table 3. The IL-10 -1082 G and A alleles were evenly

TABLE 2: Summary of study population.

Study area	Age range	Sex				Total population	
		Male		Female			
		%	*n*	%	*n*	%	*n*
Bemberi	6–15 years	48.2	63	50.8	65	35.4	128
Bandanyenje	7–14 years	51.6	118	49.4	115	64.6	233

n, number of participants.

represented in the population (51.7% and 48.3%, respectively) and occurred with similar frequency in *S. haematobium*-infected and uninfected participants. The frequency of IL-10 -819 C allele was lower in *S. haematobium*-infected participants (50%) compared to *S. haematobium*-uninfected participants (56.8%), and correspondingly, the IL-10 -819 T allele was higher in infected participants (50%) compared to uninfected participants (43.2%); however, these differences were not statistically significant (*p* = 0.342). No homozygosity for the alleles IL-10 -1082 A and TNF-α A was observed in this study population. Tumour necrosis factor-α -308 G/A alleles and genotypes had the same frequency in *S. haematobium*-infected and uninfected participants; however, the major G allele was almost four times more prevalent than the minor A allele. Interleukin-10 -1082 G/A, IL-10 -819 C/T and TNF-α -308 genotypes were examined using the chi-square test to establish whether each was associated with susceptibility to *S. haematobium* infection. However, the distribution of genotype frequencies did not differ significantly between *S. haematobium*-infected and uninfected participants (see Table 3).

Distribution of interleukin-10 -1082, interleukin-10 -819 and tumour necrosis factor-α -308 genotypes and alleles frequencies in *Schistosoma haematobium* infected and uninfected groups in Bandanyenje

Total genotype and allele frequencies in Bandanyenje are shown in Table 4. The IL-10 -1082 G/A allele frequencies were evenly distributed, where 51% had the G allele and 49% had the A allele. There was no significant difference (*p* = 0.154) in the genotype frequency distribution

TABLE 3: Distribution of the interleukin-10 -1082, interleukin-10 819 and tumour necrosis factor alpha genotypes and alleles frequencies in uninfected and infected *Schistosoma haematobium* groups in Bemberi.

Cytokine polymorphism	Allele or genotype	Frequency						*p*	χ^2	*df*
		Uninfected		Infected		Total population				
		%	*n*	%	*n*	%	*n*			
IL-10 -1082 G/A	G	51.7	31	51.5	34	51.7	125	0.979	0.001	1
	A	48.3	85	48.5	32	48.3	117	-	-	-
	GG	3.4	3	6.1	2	4.1	5	0.514	0.426	2
	GA	96.6	85	93.9	31	95.9	116	-	-	-
	AA	0	0	0	0	0	0	-	-	-
IL-10 -819C/T	C	56.8	100	50	33	55	133	0.342	0.901	1
	T	43.2	76	50	33	45	109	-	-	-
	CC	23.9	21	12.1	4	20.7	25	0.364	2.021	2
	CT	65.9	58	75.8	25	68.6	83	-	-	-
	TT	10.2	9	12.1	4	10.76	13	-	-	-
TNF-α -308 G/A	G	89.2	157	89.4	59	89.3	216	0.966	0.002	1
	A	10.8	19	10.6	7	10.7	26	-	-	-
	GG	78.4	69	78.8	26	78.5	95	0.964	0.002	1
	GA	21.6	19	21.1	7	21.5	26	-	-	-
	AA	0	0	0	0	0	0	-	-	-

IL-10, interleukin-10; TNF-α, tumour necrosis factor alpha; df, degrees of freedom.

TABLE 4: Distribution of the interleukin-10 -1082, interleukin-10 819 and tumour necrosis factor alpha genotypes and alleles frequencies in uninfected and infected *Schistosoma haematobium* groups in Bandanyenje.

Cytokine polymorphism	Allele or genotype	Frequency						*p*	χ^2	*df*
		Uninfected		infected		Total population				
		%	*n*	%	*n*	%	*n*			
IL-10 -1082 G/A	G	51.3	117	50	37	51	154	0.844	0.039	1
	A	48.7	111	50	37	49	148	-	-	-
	GG	5.3	6	0	0	4	6	0.154	2.028	2
	GA	94.7	108	100	37	96	145	-	-	-
	AA	0	0	0	0	0	0	-	-	-
IL-10 -819C/T	C	63.6	145	58.1	43	62.3	188	0.397	0.716	1
	T	36.4	83	41.9	31	37.7	114	-	-	-
	CC	31.6	36	21.6	8	29.1	44	0.183	3.392	2
	CT	64	73	73	27	66.2	100	-	-	-
	TT	4.4	5	5.4	2	4.5	7	-	-	-
TNF-α -308 G/A	G	74.6	170	75.7	56	74.8	226	0.848	0.037	1
	A	25.4	58	24.3	18	25.2	76	-	-	-
	GG	53.5	61	54.1	20	53.6	81	0.901	0.209	2
	GA	42.1	48	43.2	16	42.4	64	-	-	-
	AA	4.4	5	2.7	1	4	6	-	-	-

IL-10, interleukin-10; TNF-α, tumour necrosis factor alpha; df, degrees of freedom.

for IL-10 -1082 GA between *S. haematobium*-infected (100%) and *S. haematobium*-uninfected (94.7%) participants. Only 5.3% *S. haematobium*-uninfected participants had the GG genotype and the AA genotype was absent in both groups. Statistical analyses demonstrated that the difference in the frequency of the IL-10 -819 T/C alleles was not significant between *S. haematobium*-infected and uninfected participants ($p = 0.397$). Likewise, there was no statistical difference in the distribution of IL-10 -819 T/C genotype frequencies between *S. haematobium*-infected and uninfected participants ($p = 0.183$). The percentage frequencies of TNF-α -308 G allele (74.6% compared to 75.7%) and the TNF-α GG genotype (53.5% compared to 54.1%) were similar in *S. haematobium*-uninfected and infected participants, respectively ($p = 0.901$). Likewise, there were no significant differences in the distribution of the TNF-α A allele in *S. haematobium*-infected and uninfected participants ($p = 0.848$).

Distribution of the interleukin-10 -1082, interleukin-10 819 and tumour necrosis factor-α genotypes and alleles frequencies in uninfected and infected *S. haematobium* groups in the total study population

The total genotype allele frequencies and genotype frequencies of IL-10 -1082 G/A, IL-10 -819 C/T and TNF-α -308 G/A in total study population are shown in Table 5. Overall, there were no statistically significant differences in IL-10 and TNF-α wild-type allele distribution between *S. haematobium*-infected and uninfected participants. Interleukin-10 -1082 G allele, IL-10 -819 C allele and TNF-α -308 G allele and distributions between *S. haematobium*-infected and uninfected participants were 50.7% and 51.5% ($\chi^2 = 0.025$, $df = 1$, $p = 0.87$), 54.3% and 60.6% ($\chi^2 = 1.187$, $df = 1$, $p = 0.187$) and 82.1% and 80.9% ($\chi^2 = 0.099$, $df = 1$, $p = 0.753$), respectively. Similarly, there were no significant differences in IL-10 and TNF-α mutant allele distribution between *S. haematobium*-infected and

TABLE 5: Distribution of the interleukin-10 -1082, interleukin-10 819 and tumour necrosis factor alpha genotypes and alleles frequencies in uninfected and infected *Schistosoma haematobium* groups in the total study population.

Cytokine polymorphism	Allele or genotype	Frequency						*p*	χ^2	*df*
		Uninfected		Infected		Total population				
		%	*n*	%	*n*	%	*n*			
IL-10 -1082 G/A	G	51.5	208	50.7	71	51.3	279	0.87	0.025	1
	A	48.5	196	49.3	69	48.7	265	-	-	-
	GG	4.5	9	2.9	2	4	11	0.559	0.342	1
	GA	95.5	193	97	68	96.6	261	-	-	-
	AA	0	0	0	0	0	0	-	-	-
IL-10 819 C/T	C	60.6	100	54.3	33	59	133	0.187	1.187	1
	T	39.4	159	45.7	64	41	223	-	-	-
	CC	28.2	57	17.1	12	25.4	69	0.183	3.392	2
	CT	64.1	131	74.3	52	67.3	183	-	-	-
	TT	6.9	14	8.6	6	7.4	20	-	-	-
TNF-α 308 G/A	G	80.9	327	82.1	115	81.2	442	0.753	0.099	1
	A	19.1	77	17.9	25	18.8	102	-	-	-
	GG	64.4	130	65.7	46	64.7	176	0.872	0.275	2
	GA	33.2	67	32.9	23	33.1	90	-	-	-
	AA	2.5	5	1.4	1	2.2	6	-	-	-

IL-10, interleukin-10; TNF-α, tumour necrosis factor alpha; df, degrees of freedom.

uninfected participants. There was no significant difference in the genotype frequency distribution for IL-10 -1082 GA between *S. haematobium*-infected (97%) and *S. haematobium*-uninfected (95.5%) participants (p = 0.154). Only 4% of participants had the GG genotype which is associated with high levels of IL-10 and the AA genotype associated with low levels of IL-10 was absent in both groups. Statistical analyses also demonstrated that the difference in the frequency of the IL-10 -819 T/C genotypes was not significant between *S. haematobium*-infected and uninfected participants (p = 0.183). The TNF-α -308 GG genotype had the highest distribution (64.7%) followed by the GA genotype (33.1%), and the homozygous AA genotype had the lowest distribution (2.2%). However, there were no statistical differences in the distribution of TNF-α -308 G/A genotypes between *S. haematobium*-infected and uninfected participants (p = 0.872).

Discussion

Urogenital schistosomiasis (caused by *S. haematobium*) remains a significant threat especially for children in Zimbabwe. Host genetic factors can influence susceptibility to and severity of schistosomiasis; however, there is significant lack of studies investigating this in Zimbabwe. This study investigated the prevalence of schistosomiasis in known endemic areas of Manicaland and Mashonaland central provinces in rural Zimbabwe. It also investigated possible links between polymorphisms in the promoter regions of cytokines IL-10 and TNF-α and susceptibility to schistosomiasis, because previous studies in other parts of the world have implicated those polymorphisms in the disease susceptibility and/or immunity.

This study highlighted the importance of the disease as it established a prevalence of 26.8%, which is well above the national prevalence of 20.8% in Zimbabwe in 2012.[4] The findings may suggest a low success of current control and treatment programmes, poor coverage or the study areas being part of the remaining urogenital schistosomiasis hotspots in Zimbabwe. Similar prevalences in both boys and girls suggest overlapping unsafe water-related chores and/or activities.

Susceptibility to schistosomiasis has been associated with genetic components, apart from socio-economic, environmental and ecological factors.[22] Thus, certain genotypes are thought to be more prone to schistosomiasis than others. The genes *TNF-α* and *IL-10* have attracted considerable attention as possible contributors to susceptibility or resistance to infectious, non-communicable, immune-mediated and autoimmune diseases.[20] Studies have demonstrated an association between different allelic variants and differential production of IL-10. Polymorphism of the promoter region has led to different haplotypes with different levels of IL-10 production, that is, 'high' IL-10 producer haplotype (GCC/GCC), 'intermediate' producer haplotypes (GCC/ACC, GCC/ATA) and 'low' producer haplotypes (ATA/ATA, ACC/ATA, ACC/ACC), where positions 1082, 819 and 592 are represented.[18] Overall, high production is hinged on having a G in the -1082 position independent of the -819 and -592 polymorphisms.

High IgE and low IgG4 antibodies against a variety of schistosome antigens have been associated with resistance. Immunoglobulin G4 (IgG4) is thought to inhibit the actions of IgE. Interleukin10 is associated with IgG4 production and blocking the receptors of IL10 in experimental mice models has been linked to the development of significant protection against re-infection after treatment.[18,23] Interleukin10, predominantly produced by CD4+ lymphocytes, obstructs the development of acquired resistance, reduces morbidity and prolongs survival in schistosomiasis.[12,15] The 'low' ATA/ATA haplotype has been associated with increased circulating eosinophil counts; worm-specific IgG1, IgG2b and IgE levels; and enhanced Th1, Th2 and Th17 responses,

which are key in conferring immunity against schistosomes.[15,18] Lower levels of IL10 thus correspond to low susceptibility to schistosomiasis and development of immunity after treatment. After correction for sex, age and infection status at study onset, high levels of parasite-specific IL-10 were recognised as a risk factor for re-infection in a study conducted in Gabon.[14]

Tumour necrosis factor alpha, a potent immunomediator and pro-inflammatory cytokine, is generally produced in the early inflammatory stages of infection. It has been linked with liver granulomas/fibrosis and egg-laying of the parasite.[24,25] Tumour necrosis factor alpha and INF-γ are indicators of a Th1 response and are often elevated in acute schistosomiasis. Contradictions can be observed from scientific reports as some conclude that TNF-α is protective against severe disease, whilst others noted that high levels aggravate disease in *S. mansoni*.[16] The increased frequency of the rare allele TNF-308A (TNF2) has been reported in autoimmune disorders, such as rheumatoid arthritis, systemic lupus erythematous and coeliac disease. Relative to the more common TNF1 (TNF-308G) allele, the TNF2 allele is a more powerful transcriptional activator, and thus more TNF-α is expected in individuals with TNF2 allele(s).[20] The TNF2 allele can then be hypothesised as increasing the resistance of the host to local infection (by increasing local production of TNF at the infection site). The limiting of successful infection after entry of cercariae, is also associated with increased risk for severe pathology and other chronic, inflammatory or autoimmune diseases.[26] In Kenya, TNF-308 promoter polymorphism allele 2 has been associated with early childhood mortality and malaria morbidity. Tumour necrosis factor 2 has also been associated with pathogenesis of asthma, peptic or duodenal ulcers, coronary heart disease and angina.[27]

We therefore hypothesised that the IL-10 -1082 A allele and TNF-α 308 A allele, as well as genotypes with these alleles, would be associated with reduced susceptibility to schistosomiasis in our study population. The frequencies of IL-10 -1082 and TNF-α 308 alleles or genotypes were similar to those observed in other studies. The frequency of G allele (TNF-α 308 G/A) was above 80% as was expected in African people.[26] In addition, IL-10 -1082 G/A genotypes with at least one G had similar frequencies as was observed in other studies.[28,29] However, contradictory to our initial hypothesis of these genotypes associating with susceptibility to schistosomiasis, we observed no difference in frequency of these genotypes between *S. haematobium*-infected and uninfected participants. The findings further highlight the complexities in host immune–parasite interactions. As the study was not controlled, many confounding and predisposing factors (such as poverty, possible under-nutrition, high prevalence, high-contaminated water contact, co-infections amongst other risk factors) might have eroded the effects of host genetic factors that were expected. Studies have shown that schoolgoing children are at a higher risk than other age groups.[30] In the same study, Ismail et al.[30] also demonstrated that high water contact associated with communal and agricultural (irrigation) lands was linked with high prevalence rates.[30,31] Other factors associated with prevalence and high infection intensities in endemic areas are gender, occupation, female household head's education level, religion, socio-economic status and house location.[32] The highlighted factors have been found to influence a person's contact with infested water.[32] Thus, the high prevalence noted and the lack of proper control for the highlighted factors in this study could have eroded the minor effects of host genetic factors. There is probably a need to have larger sample sizes and correction for the suggested confounding and predisposing factors in future studies. Controlled studies may also highlight the link between IL-10 production levels to susceptibility to schistosomiasis. The study was also limited by the number of children who were willing or able to provide adequate blood sample for genotyping.

Conclusion

The findings failed to demonstrate any significant relationship between host genetic factors considered (i.e. IL-10 -1082 G/A, IL-10 -819 C/T and TNF-α -308 G/A SNPs) with susceptibility to urogenital schistosomiasis. The prevalence of schistosomiasis is still in the moderate range and is similar in boys and girls. Future studies are recommended to include investigations of possible links between the SNPs and morbidity or pathology.

Acknowledgements

The authors would like to thank the laboratory and field mates and technicians at the Department of Biochemistry, University of Zimbabwe and School of Laboratory Medicine, University of KwaZulu-Natal for their assistance in conducting this study.

Competing interests

The authors have declared that no competing interests exist.

Authors' contributions

A.M., A.V., J.M. and T.M. developed the field study design, conducted field and sampling work and immunoassays and analysed the data. T.M. and J.M. supervised the work. All authors contributed to the final manuscript.

Funding information

Funding for this study was provided by the Malaria Training and Research Capacity Building in Southern Africa (grant number: NIH/FIC2D43TW001587-06A2), Schistosomiasis Control Initiative, United Nations Children's Fund and World Health Organization.

Data availability statement

Data are available from the corresponding author subject to requests complying with the terms and conditions set out by the authors.

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CHAPTER 3

Formatted according to African Journal of Laboratory Medicine guidelines were it has been provisionally accepted. The draft has been resubmitted after the first round of corrections.

TNF-α and IL-10 -819 T>C Single Nucleotide Polymorphisms Effects on Urogenital Schistosomiasis in Pre-school Children in Zimbabwe

Amos Marume[1, 2*], Arthur Vengesai[1, 3], Theresa Chimponda[3], Caroline Mushayi[3], Jaclyn Mann[1] and Takafira Mduluza[1, 3]

[1]Department of Infection Prevention & Control, School of Laboratory Medicine & Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa.

[2]Paraclinical Department, Faculty of Veterinary Sciences, University of Zimbabwe, P.O. Box MP 167, Mt Pleasant, Harare, Zimbabwe.

[3]Department of Biochemistry, Faculty of Science, University of Zimbabwe, P.O. Box MP 167, Mt Pleasant, Harare, Zimbabwe.

*Corresponding author: Amos Marume (stafirejee@gmail.com)

ABSTRACT

Background: Knowledge gaps exist between host genetic factors and susceptibility to schistosomiasis a disease with serious morbidity and pathology.

Objectives: To determine cytokine levels and single nucleotide polymorphisms of TNF-α (rs1800629) and IL-10 (rs1800871) and their possible impact on susceptibility to schistosomiasis in pre-school age children.

Methods: Urogenital schistosomiasis was diagnosed using the urine filtration method, while sandwich ELISA was used for cytokine levels determination. The survey was done in August of 2015 and reinfection levels post treatment were done at 3, 6 and 12 months. Amplification Refractory Mutation System Polymerase Chain Reaction with visualization on 2% agarose gel electrophoresis was used for genotyping.

Results: Urogenital schistosomiasis prevalence was found to be 10.5 % (59/563), where 50.8% of children were male and 49.2% were female, and reinfections were detected in only 6 children at 3 months and only 1 was reinfected at 12 months. There were no significant differences in TNF-α-308 G/A allele or genotype frequencies, respectively, between the *S. haematobium* infected and uninfected participants (p = 0.360 and 0.279). However, no children with the IL-

10 -819 TT genotype (associated with low production of IL-10) had schistosomiasis. The TNF-α GG genotype corresponded with significantly lower TNF-α levels when compared with the GA or AA genotypes ($p<0.05$), and TNF-α levels were significantly lower in infected children compared to uninfected children ($p<0.05$).

Conclusion: Higher TNF levels and lower IL-10 levels are potentially protective against schistosomiasis infection. IL-10 -819 TT genotype is potentially protective against infection through its association with lower IL-10 levels.

Key words: *S. haematobium*, polymorphisms, cytokines, susceptibility, protective immunity

INTRODUCTION

Pre-school children from poor and endemic regions like sub-Saharan Africa are at high risk of schistosomiasis. The region has poor hygiene and sanitation, conditions that facilitate *Schistosoma* thriving. In the endemic regions people living in both rural and urban centers are at risk of getting infected with schistosomes, as a result of using of open water sources infested with intermediate snails harboring the human host infectious stage [1]. Most of the people that are at risk of schistosomiasis worldwide have low awareness levels of the disease [2,3].

Urogenital schistosomiasis has been described as a significant public health threat for pre-school age children (≤5 years); often they have higher infection burdens, and show serious morbidity and pathology relative to school-age children [4]. Thus pre-school children in endemic areas are regarded as a high-risk group and needing inclusion in mass drug administration programs [1]. Praziquantel treatment despite some few known side effects is acceptable, safe and effective in all age ranges; treatment leads to 91% reduction in prevalence as well as 93% decrease in infection intensity [4]. Praziquantel causes rapid influx of Ca^{2+} accompanied by an intense muscular paralysisis and is known to remove the immune-suppressive effects of adult worms as well as lead to expression of worm antigens as a result of dying worm degeneration [5]. Thus praziquantel aids in immunity development.

Most endemic regions of Zimbabwe has prevalences that range between moderate to high risk according to WHO guidelines [6,7]. Thus mass praziquantel administration should be done at least once every two years [7]. Morbidity and mortality of schistosomiasis is well recognized to be a consequence of the host immune system [5]. People in endemic areas are known to develop protective immunity against reinfections, which offers hope for possible vaccines [4]. The

immunology of schistosomiasis is therefore of great significance in management, control and possibly eradication of infection.

Research, control strategies and treatment guidelines of schistosomiasis in children under 5 years of age are reported to have lagged behind those in other age groups [5]. Thus significant knowledge gaps exist for the pre-school age group in relation to prevalence, morbidity, mortality, immunology, immunopathology, treatment effectiveness and safety, and impact of host genetic factors.

We therefore assessed cytokine levels as well as cytokine genotypes in pre-school children at risk of schistosomiasis. TNF-α and IL-10 have been associated with susceptibility and other key immune responses in many diseases including those caused by helminths [8–10]. Therefore we associated rs1800629 and rs1800871 promoter region polymorphisms, as well as TNF-α and IL-10 levels, with susceptibility to urogenital schistosomiasis among pre-school children. Reinfection levels post-treatment were also assessed at 3, 6 and 12 months in follow-up surveys.

MATERIALS AND METHODS

Ethical considerations

The study was registered and ethically approved by the Zimbabwe's ethics board for bio-medical research (the Medical Research Council of Zimbabwe - MRCZ/A/1710). In addition, community leaders, Provincial Medical Director and District Medical Officer also granted permission to conduct the study in Madziva, Shamva. The children agreed to participate in the study and their parents/guardians signed informed consent forms allowing their participation. The questionnaires about medical history and informed consent form were translated into relevant vernacular languages. The researchers also took time to ensure the guardians or parents understood the forms. The children and parents were educated on the aims, risks and benefits of the study. Participation was voluntary and participants were free to withdraw from the study at any time.

Study population and sample

The study population was previously described Midzi *et al* (2014) [11]. The survey of urogenital schistosomiasis prevalence was conducted among 563 children aged between 1 – 5 years through collection of urine specimens. Genotyping was done on 268 children who were willing and were able to provide adequate blood. Convenience sampling was used, the sample was

several times larger than 50 children per centre an estimate made by Midzi *et al* (2014) [11]. The children were permanent residents of Madziva area in Shamva district, Mashonaland Central province, Zimbabwe. The area was selected for this study based on its high annual rainfall, a condition conducive for *Schistosome* species and leading to high schistosomiasis endemicity [12]. The study was a cross sectional study, which was part of an ongoing longitudinal study on urogenital schistosomiasis for the 1-5 age group (see funding section below). Participants infected with *S. mansoni* as determined by the Katz *et al*. (1972) [13], were excluded from this study. The survey was done in August of 2015 and reinfection levels post treatment were done at 3, 6 and 12 months.

Detection of *S. haematobium*

Schistosoma haematobium was diagnosed by the microscopic examination of urine specimens for the presence of parasite eggs using the urine filtration technique [14]. In brief, urine specimens were collected from willing participants for three consecutive days. For diagnosis 10 ml of urine was filtered through a nitrile filter membrane. The membrane was stained with iodine and examined under a light microscope. The same procedure was repeated on three consecutive days in order to prevent misdiagnosis due to day-to-day variation of egg excretion [15]. If positive, the number of eggs in the entire sample of 10 ml of urine was counted and recorded as light infection if 1 - 10, moderate infection if 11 - 49 or heavy infection if > 50 eggs. Participants who were *S. haematobium* positive were treated with a single dose of praziquantel (40 mg per kilogram of body weight). Bread and orange juice were given as supplementary food in order to enhance absorption as well as to alleviate the nauseating effects of praziquantel. Stool samples were collected once for *S. mansoni* diagnosis. Urine collection procedures and the diagnosis were repeated at 3, 6 and 12 months to assess reinfection levels post treatment with praziquantel.

Blood collection

Approximately 5 ml blood was collected into Ethylenediamine tetraacetic acid (EDTA) tubes. The tubes were centrifuged at 3000 rpm for 10 minutes using a rotofix centrifuge. Plasma was collected and used to measure the levels of systemic cytokines. Host DNA was extracted from whole blood for genotyping using the Qiagen FlexiGene DNA extraction kit (Qiagen FlexiGene® DNA Handbook, Qiagen, Germany).

Genotyping

The TNF (rs18006290) and IL10 -819T>C (rs1800871) polymorphisms were determined using amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). The primers used were from Inqaba Biotechnology, Pretoria, South Africa (Table 1). Primers specific for wild type genotype (1ul) and mutant genotype (1ul) were separately mixed with 1ul (10 mM) generic primer, 0.5 ul 10 mM forward and 0.5 ul 10 mM reverse internal control primers (human growth hormone), 12.5 ul quick load Taq 2x master mix (New England Biolabs, Ipswich, MA USA 01938-2732) and 4.5 ul of sterile, nuclease-free water (New England Biolabs). Five microliters of the template DNA was added to the master mixes and loaded onto a thermocycler (PXE 0.2 thermocycler Thermo Electron Corporation, Waltham, MA USA 02451). IL-10 -819 T>C alleles were amplified using the following conditions: 1 minute denaturation step at 95°C; 10 cycles of 15 seconds at 95 °C, 50 seconds at 65 °C, and 40 seconds at 72 °C; and 20 cycles of 20 seconds at 95 °C, 50 seconds at 59 °C and 30 seconds at 72 °C, followed by cooling at 4 °C. TNF polymorphisms were amplified using the following conditions: 1 minute denaturation step at 95°C; 10 cycles of 15 seconds at 95 °C, 45 seconds at 60 °C, and 35 seconds at 72 °C; 25 cycles of 20 seconds at 95 °C, 45 seconds at 60 °C, and 45 seconds at 72 °C, followed by cooling at 4 °C. ARMS-PCR amplicons were analysed by electrophoresis on 2 % agarose gel stained with ethidium bromide. The gels were viewed on a UV trans-illuminator to score the presence or absence of the cytokine gene polymorphisms.

TNF- and IL-10 determination

The levels of the cytokines were measured by indirect enzyme linked immunosorbent assay (ELISA) using MABTECH, 3510-1H-6 kits, according to the manufacturer's instruction (Mabtech AB, Büro Deutschland, Germany). The assay was performed in duplicate and the data was averaged.

Statistical analysis

Genotype frequencies of the TNF (rs1800629) and IL-10 -819T>C (rs1800871) between *S.* haematobium infected and uninfected participants were compared by Chi-square test. The Hardy-Weinberg equilibrium was assessed for TNF (rs1800629) and IL-10 -819T>C (rs1800871) S. haematobium infected and uninfected participants groups by Chi-square. Genotype data was analysed with the software IBM SPSS Statistics for Windows, Version 19.0. (IBM Corp., Armonk, NY, USA). Cytokine levels were compared between infected and

uninfected participants using unpaired t-tests, and between different genotypes and ANOVA was used to compare between light, moderate and heavy infection groups. P-values < 0.05 were considered statistically significant.

RESULTS

Prevalence of *S. haematobium* infection

There was no significant difference (p = 0.823) between boys (n = 294) and girls (n = 269) recruited. The prevalence of *S. haematobium* was 10.5 % (n=59/563), with an even distribution between males (50.8 %) and females (49.2 %) (p = 0.82).

Table 1: List of primers used in genotyping

Cytokine	Position	Mutation	Primer sequence (5'-3')	Fragment
rs1800871	-819	C→T	Wild type: CCC TTG TAC AGG TGA TGT AAC Mutant: CCC TTG TAC AGG TGA TGT AAT Generic: AGG ATG TGT TCC AGG CTC CT	233 bp
rs1800629	-308	G→A	Wild type: AGG TTT TGA GGG GCA TGG Mutant: AGG TTT TGA GGG GCA TGA Generic: CAG CGC AAA ACT TCC TTG GT	267 bp

TNF and IL-10 genotypes are not associated with infection status or intensity

All genotypes of rs1800629 and rs1800871 were represented in the population, and consistent with previous literature, the TNF-α-308 GA genotype and IL-10 CT genotype were the most common. None of the genotypes studied showed significant differences between the infected and uninfected groups (Table 2). When stratified the data by infection intensities again there no significant differences between the infected and uninfected groups. (Table 3). Interestingly, 10 children had the IL-10 TT genotype (associated with low production of IL-10) and none of these were infected, raising the hypothesis that the TT genotype might be protective against *S. haematobium*, although this was not statistically significant.

It was found that the rs1800871 single nucleotide polymorphism (SNP) was in line with the Hardy-Weinberg equilibrium in uninfected participants (Table 2). However the rs1800629 SNP was not in line with Hardy-Weinberg equilibrium in both *S. haematobium* infected and uninfected participants (Table 2).

Table 2: Genotype distributions of TNF (rs1800629) and IL-10 -819T>C (rs1800871) between *S. haematobium* infected and uninfected groups.

SNPS	Uninfected	Infected	X^2	P value	Uninfected		Infected	
					X^2 for HWE	P value for HWE	X^2 for HWE	P value for HWE
rs1800629			2.276	0.320	1595.664	0.000	70.311	0.000
GG	21 (8.7)	3 (12.5)						
AG	158 (65,3)	18 (75)						
AA	63 (26.0)	3 (12.5)						
rs1800871			0.951	0.622	6.781	0.034	_	_
CC	12 (24.5)	2 (40.0)						
CT	32 (65.3)	3 (60.0)						
TT	5 (10.2)	0 (0)						

Table 3: Genotype distributions of TNF (rs1800629) and IL-10 -819T>C (rs1800871) grouped according to *S. haematobium* infection intensity.

SNP	No Infection	Light Infection	Moderate Infection	Heavy Infection	X^2	P-Value
rs1800629					2.847	0.828
GG	21 (8.7)	2 (11.8)	1 (16.7)	0 (0)		
AG	158 (65.3)	13 (76.5)	4 (66.7)	1 (100)		
AA	63 (26.0)	2 (11.8)	1 (16.7)	0 (00		
rs1800871					1.131	0.889
CC	12 (24.5)	1 (33.3)	1 (50)	0 (0)		
CT	32 (65.3)	2 (66.7)	1 (50)	0 (0)		
TT	5 (10.2)	0 (0)	0 (0)	0 (00		

Cytokine levels were associated with cytokine genotypes and infection

The TNF-α GG genotype corresponded with significantly lower TNF-α levels when compared with the GA or AA genotypes (Figure 1 a) ($p<0.05$), and TNF levels were significantly lower in infected children compared to uninfected children (Figure 1 b). The IL-10 TT genotype corresponded with significantly lower IL-10 levels when compared with CC (Figure 1 c). IL-10 levels were significantly lower in uninfected children compared to infected children (Figure 1 d) (unpaired t-test; $p=0.0007$).

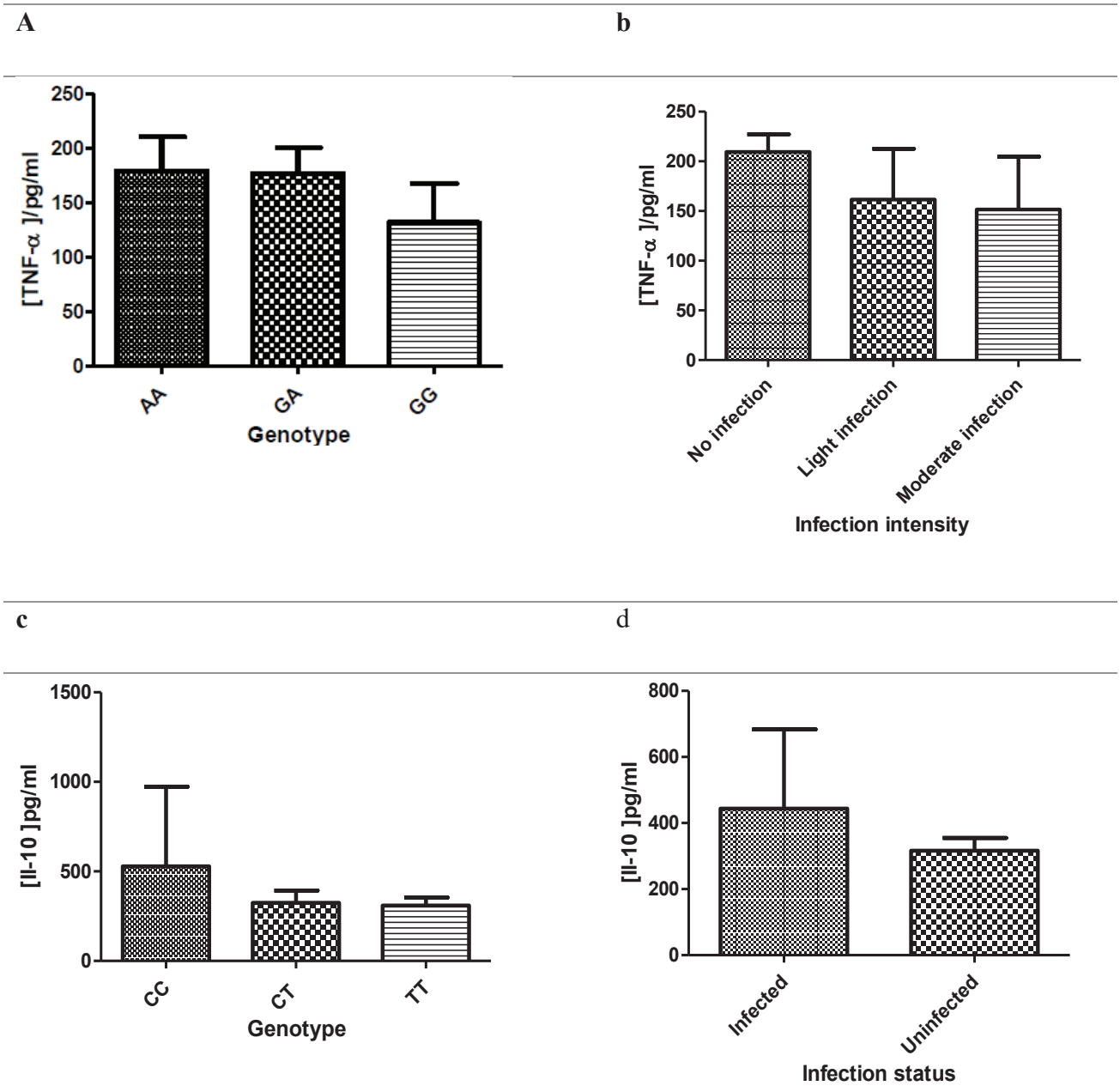

Figure 1: Genotypes, cytokine levels and infection status

a - Genotypes and TNF-α production (mean level for AA is 179.3 ± 31.2 pg/ml n=34, GA is 176.8 ± 23.8 pg/ml n=98 and GG is 131.6 ± 36 pg/ml n=14); b - TNF-α levels against infection intensity (239 ± 71.6 pg/ml n=23 and 210 ± 17.6 pg/ml n=286); c - Genotypes against IL-10 plasma levels (CC - 528.5 pg/ml, CT - 323.8 pg/ml and TT – 309.4 pg/ml) d - IL-10 plasma levels against infection status (452.5 pg/ml and 376.4 pg/ml).

Reinfection

Reinfections at 3 months post-treatment were detected in 10.2% (6/59) of children that were previously infected. At 6 months there were no-reinfections observed and only one child was reinfected after one year post-treatment.

DISCUSSION

The study describes results from a survey on prevalence of urogenital schistosomiasis in preschool children and impact of single nucleotide polymorphisms of TNF-α-308 G/A and IL-10-819 C/T gene promoter regions on cytokine levels, susceptibility to *S. haematobium* infections and treatment outcomes. The results highlighted and supported existing evidence on schistosomiasis prevalence levels (10.5%) in 1-5 years old males and females and also supported the possible effectiveness in mass drug administrations and immune development facilitation effects of praziquantel treatment as only 6 were reinfected by 1 year post-treatment. The study showed that cytokine levels were different depending on the individual's genotype: the IL-10 -819 CC genotype corresponded with the highest levels of IL-10, while the CT and TT genotypes corresponded with moderate and low levels of IL-10, respectively; and the TNF-α-308 AA genotype corresponded with the highest, GA with moderate to high and GG with low TNF-α levels. A major finding was that TNF-α and IL-10 levels predict susceptibility to *S. haematobium* infection, with higher TNF-α and lower IL-10 levels conferring protection. Interestingly, all 10 children who had the IL-10 TT genotype (associated with low production of IL-10) were uninfected, suggesting that this genotype may confer a protective effect, although this was not supported by statistical tests. Higher levels of IL-10 have also previously been linked with possible *S. haematobium* susceptibility [16]. IL-10 has immunomodulatory effects which may explain the susceptibility to schistosome infections - higher levels of IL-10 have also been demonstrated to be protective against serious morbidity [16,17]**.** Higher levels of the pro-inflammatory cytokine (TNF-α) could aid in immunity development against schistosomes as is suggested by the findings of this study. Also the findings of the present study show that intensity of infection was somewhat linked to TNF-α levels as those with moderate levels of TNF-α had light infection (1-10eggs/10ml of urine) and those with low levels had moderate infection (11-49eggs/10ml), although this was not statistically significant?. This supports the hypothesis that TNF-α is also helpful in limiting the proliferation of the parasite.

The allele or genotype frequencies were as expected in Africans - IL-10 819 CT genotype frequencies were above 50% and TNF-α-308 GA genotype frequencies were above 80% [18,19].

The relationship between cytokine levels and genotypes was also consistent with that in previous studies [20]. IL-10 -819 CC genotype corresponded to high levels of plasma IL-10 while CT to moderate and TT to low levels of IL-10 levels. For TNF-α-308 AA genotype corresponding to high, GA – moderate to high and GG – low plasma levels of TNF-α [20]. IL-10 promoter SNPs -1082G/A, -819C/T and -592C/A, are involved in differential IL-10 gene transcription and hence differential IL-10 production [20]. The findings highlighted a general lack of association between any genotype with urogenital schistosomiasis however the data pointed to a possible protective effect of IL-10 -819 TT. Given that actual cytokine levels, rather than the SNPs studied here, show an association with susceptibility to *S. haematobium* infection, the findings highlight complexities that may exists in the expression of those genes and/or that additional SNPs play an important role in the expression of these cytokines. Although no clearly significant association was demonstrated in this study, IL-10 haplotypes GCC and GTA were previously linked to high/moderate IL-10 production were demonstrated to be associated with schistosomiasis [20]. This may highlight the need for a larger sample size to demonstrate the suggested link between genotypes and susceptibility or the need to identify and study other polymorphisms that affect expression of IL-10 and TNF-α.

Peripheral blood mononuclear cells from subjects with schistosomiasis have been shown to produced 8 fold greater levels of egg antigen–driven TNF-α leading to a 99 fold greater mean TNF-α : IL-10 ratio, relative to negative controls [17]. Some studies have established a positive relationship between infection intensity and TNF-α production [21,22]. IL-10 elevation in schistosome infections was also demonstrated with some authors suggesting both TNF-α and IL-10 as a possible biomarkers in schistosomiasis [21–23]. Thus the high levels of IL-10 in infected individuals could have been a result of the infection rather than the cause of infection. However, there are previous reports that high levels of IL-10 predispose to reinfection [8–10] and we found that no individuals with the IL-10 TT genotype (which was also associated with lower IL-10 levels) were infected. Taken together these observations support that the association between IL-10 levels and schistosomiasis in our study is due to higher IL-10 levels predisposing to infection and lower levels conferring protection. Some studies detected elevated levels of TNF-α in infected individuals relative to uninfected controls especially in acute schistosomiasis [24]. The findings of this study however contradicts those observations as low levels of TNF-α were associated with infection, which actually support the inferences that higher levels TNF-α and indeed lower levels of IL-10 are actually protective against *S. haematobium*.

In this study most children did not get re-infected after 3, 6 and 12 months suggesting possible efficacy of praziquantel mass drug administrations in elimination of schistosomiasis efforts. The few that got possible reinfection fall in the category of moderate to high production of TNF-α (individuals with the TNF-α GA and AA genotypes), which appears contradictory to our observation that high levels of TNF-α are negatively associated with infection. The effects of praziquantel treatment on immunological profiles and immunity to reinfection was reported to be similar in all age groups of children [4]. However, TNF-α was shown to play a complex role in propagating the intramammalian stages of the schistosome life cycle - an effect that helps schistosome survive in the host [25]. This might explain the observed few reinfections. Alternatively some of the "reinfections" might represent treatment failure cases, particularly since 5/6 cases were observed at 3 months post-treatment, and high levels of TNF-α may have predisposed the individuals to treatment failure. Some studies suggest that IL-10 blocks the development of immunity after praziquantel treatment thus re-infection is better prevented when praziquantel treatment is administered in individuals with low or blocked IL-10 [26]. Some researchers have reported contradictory findings for example intermittent treatment of *S. japonicum* or *S. mansoni* infection has been shown to increase the risk for respective disease in some individuals [17]. The re-infections could also be explained by the fact that the participants were inhabitants of an endemic areas and praziquantel administrations were not coupled with other key interventions like killing of intermediate hosts and provision of safe water. Thus the study could not conclusively explain the re-infections. The significant reduction of prevalence over the 12 months could help support sustained community based praziquantel administrations. Such efforts can be reasonably expected to significantly reduce the schistosome burdens in endemic areas.

The prevalence of 10.5% though still high may suggest effectiveness of prior mass drug administration and health education efforts as it is below 20.8% (nationally reported urogenital schistosomiasis prevalence in 2012) [27]. In 2011, the province (Mashonaland Central) had overall prevalence of 26.1 (23.46–28.90) and Shamva was also categorized among the top districts with heavy intensities (53.2%) of *S. haematobium* infections [12]. More efforts like snail control, preventative chemotherapy and improvement of hygiene and sanitation are still required as these have at some point sustained prevalence below 10% in areas like Hippo Valley Sugar Estates, Zimbabwe [28]. The observed lack of differences in prevalence of schistosomiasis in males and females is in contrast to what was established by major surveys conducted in Zimbabwe previously [12,29]. In those primary school surveys females consistently

had lower prevalence of infection compared to their male counterparts. The lack of differences between boys and girls can be attributed to many often overlapping activities or chores like swimming, washing and fetching water from unprotected sources. The major factor is also that the water contact of pre-school children is parent or guardian related, thus no differences could be expected. Gender differences in activities often starts to vary at around age 6 thus similar prevalence of infection is expected in children <6 years of age and gender differences may emerge thereafter [30].

Limitations

The prevalence of schistosomiasis was low which could have limited the ability to highlight association of any of the studied genotypes with susceptibility to *S. haematobium*. Those the sample was larger than the recommended 50 participants per centre resources limited the ability to have more participants which could have helped in establishing rare phenomena. The results could also have been affected by the fact that we had a few individuals who had heavy infection intensities. Also better results on possible association of urogenital schistosomiasis and genotypes could have been obtained if all the recruited children were willing and able to provide adequate blood for genotyping. The highlighted limitations could be managed by combining the genotyping data with other similar data from children even of another age group and analyse for possible associations. Future studies are recommended to include many cytokines and all possible single nucleotide polymorphisms known to influence cytokine gene expression.

CONCLUSION

Prevalence of urogenital schistosomiasis among boys and girls was indistinguishable statistically. For TNF-α-308 GA genotype had the highest frequency and AA, GA and GG genotypes were associated with high, moderate to high and low production of TNF-α. TNF-α -308 G/A and IL-10 -819 C/T polymorphisms were not significantly associated with susceptibility to *S. haematobium* infection, although no individuals with the IL-10 TT genotype (n=10), which is associated with lower IL-10 levels were infected in this study. Higher TNF-α and lower IL-10 levels were negatively associated with infection and are suggested to confer protection against schistosomiasis. Praziquantel helped in significantly reduce prevalence of schistosomiasis in pre-school children staying in an endemic area.

The obvious implications of the findings include the need for continued efforts (mass drug administrations, better sanitation and hygiene and health education and promotion) to reduce

prevalence to zero among children in endemic areas of Zimbabwe. Other implications could include immune-based interventions that encourage TNF-α but limit IL-10 production as this will promote immunity against *S. haematobium*, with possible risks associated with the resultant proinflammatory environment.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflict of interest

The authors declare no conflict of interest.

Author Contribution

AM, TC, AV, CM and TM developed the field study design, conducted field and sampling work, immunoassays and analysed the data. TM and JM supervised the work. All authors contributed to the manuscript.

Acknowledgements

We would like to thank the community of Madziva, Shamva district for their participation and support in the study. We also thank the Biochemistry staff, University of Zimbabwe and the infectious disease group for their assistance in the fieldwork.

Funding

NIH/FIC2D43TW001587-06A2 Malaria Training and Research Capacity Building in Southern Africa. Welcome Trust and Thrasher Medical Fund, Schistosomiasis Control Initiative, UNICEF and WHO.

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CHAPTER 4

Additional findings: Risk factors of schistosomiasis in selected endemic areas of rural Zimbabwe

4.1. Summary of the study population in total

The following results are from additional analysis of combined data for both preschool (1 – 5 years) and primary school (6 – 15 years) children. The data analysed was generated as outlined in chapter 2 and 3 methodology sections. Table 4.1 shows the summary of the total population after combining all the children (preschool and school age children).

4.2. Statistical analysis

Genotype frequencies of the TNF (rs1800629), IL-10 -819T>C (rs1800871) and IL-10 -1082A>G (rs180096), between *S. haematobium* infected and uninfected participants were compared by Chi-square test. The Hardy-Weinberg equilibrium was assessed for TNF (rs1800629), IL-10 -819T>C (rs1800871) and IL-10 -1082A>G (rs180096), S. *haematobium* infected and uninfected participants groups by Chi-square. Genotype data was analysed with the software IBM SPSS Statistics for Windows, Version 19.0. (IBM Corp., Armonk, NY, USA). Where it was not possible to determine the Hardy-Weinberg equilibrium using SPSS equations shown below were used for the assessment. P-values < 0.05 were considered statistically significant.

$$x^2 = \sum \frac{(O - E)^2}{E}$$

$$df = (r - 1)(c - 1)$$

Where X^2 = Chi square

df= degrees of freedom

r= number of columns

c= number of raws

O= observed value

E= expected value

∑= the sum of

4.3. Results

A total of 924 participants, 485 (52.5 %) males and 439 (47.5 %) females and 354 (38.3 %) primary school aged children and 570 (61.7 %) pre-school aged children were recruited for the study. The demographic of 158 (17.1 %) *S. haematobium* infected participants and 766 (82.9 %) uninfected participants are shown in Table 4.1. No significant difference was found between the *S. haematobium* infected and uninfected groups in terms of gender (x^2=0.114, p=0.735). Significant difference was found between *S. haematobium* infected and uninfected groups in terms of study site (x^2=38.943, p=0.000) and age group (x^2=38.377, p=0.000). The older the child the higher the risk of getting infected by *S. haematobium* (10.5% in 0-5 year olds; 24.0% in 6-10 year olds and 30.7% in 11-15 year olds; p = 0.000).

Table 4.1: Demographic and clinical characteristics of *S. haematobium* infected and uninfected individuals in the total study population

Variable	**Uninfected**	**Infected**	**X^2**	**P value**
Site			38.943	0.000
Bemberi	86 (11.2)	35 (22.2)		
Bandanyenje	173 (22.6)	60 (38.0)		
Madziwa	507 (66.2)	63 (39.9)		
Gender			0.114	0.735
Male	404 (52.7)	81 (51.3)		
Female	362 (47.3)	77 (48.7)		
Age group			38.377	0.000
Primary school aged children (6-13)	259 (33.8)	95 (60.1)		
Pre-school aged children 5>	507 (66.2)	63 (39.9)		

n (%)

We observed that the GG, AG, and AA genotypes of TNF (rs1800629) showed significant differences between *S. haematobium* infected and uninfected groups (x^2=26.089, P=0.000; table 2) and the CC, CA genotypes of IL-10 -819T>C (rs1800871) and IL-10 -1082 A>G (rs1800896) revealed no significant differences between the infected and uninfected groups with p-values of 0.092 and 0.559 respectively. However, we found all the SNPs TNF (rs1800629), IL-10 -819T>C (rs1800871) and IL-10 -1082 A>G (rs1800896) were not in line with Hardy-Weinberg equilibrium in both the infected and uninfected groups.

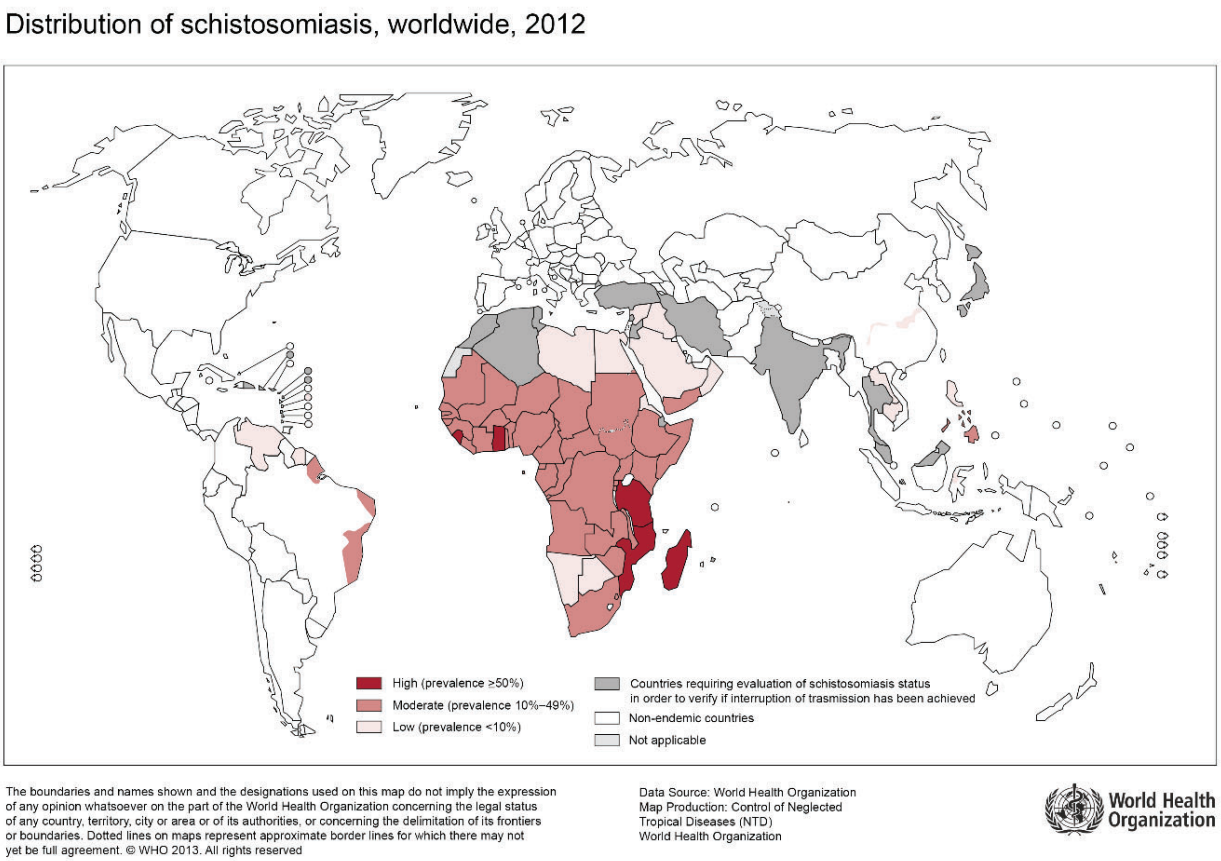
Table 4.2: Genotype distributions of TNF (rs1800629), IL-10 -819T>C (rs1800871) and IL-10 -1082 A>G (rs1800896) between *S. haematobium* infected and uninfected groups

SNPS	Uninfected	Infected	X^2	P value	Uninfected		Infected	
					X^2 for HWE	P value for HWE	X^2 for HWE	P value for HWE
rs1800629			26.089	0.000	992.787	0.000	741.804	0.000
GG	151 (34.1)	49 (51.6)						
AG	225 (50.8)	41 (43.2)						
AA	67 (15.1)	5 (5.3)						
rs1800871			4.776	0.092	38.239	0.000	20.814	0.000
CC	69 (27.5)	14 (18.7)						
CT	163 (64.9)	55 (73.3)						
TT	19 (7.6)	6 (8.0)						
rs1800896			0.342	0.559	383.52	0.000	536.68	0.000
GG	9 (4.5)	2 (2.9)						
GA	193 (95.5)	68 (97.0)						
AA	0 (0)	0 (0)						

n (%)

4.4. Discussion

The findings highlight that while age and geographical location were risk factors to schistosomiasis gender was not. The average prevalence of schistosomiasis among the study participants was 17.1 % (158/924). Based on the global distribution map (Figure 4.1) of 2012, Zimbabwe is in the moderate range for schistosomiasis infection (10 – 49% prevalence) (ECDC, 2014). Thus, the findings of this study not only support the categorization but also help highlight the strong need for more concerted efforts in the fight against schistosomiasis.



Source: http://gamapserver.who.int/mapLibrary/Files/Maps/Schistosomiasis_2012.png

Figure 4.1: Global distribution of schistosomiasis

In endemic areas almost every one of the long-term residents will be infected with schistosomes at some point in their life. In regions with typical transmission patterns school going children carry 60 – 80% of the infection burden as compared to adults (Colley *et al.*, 2014). Children are at risk of schistosomiasis due to many activities that brings the in contact with open water sources such as dams and rivers and their immature immunological status (Chisango *et al.*, 2019). Age, gender, occupation, female household head's education level, religion,

socioeconomic status, agricultural activities, water sources and house location are some of the factors that have been found to influence a person's contact with infested water (Yang *et al.*, 2009; Colley *et al.*, 2014; Chadeka *et al.*, 2017; Mewabo *et al.*, 2017; Hajissa *et al.*, 2018). Studies in Nigeria also highlighted swimming and drinking contaminated water as the most important risk factors followed by washing clothes and taking baths in rivers (Oluwasogo and Fagbemi, 2013). A study in Kano State, Nigeria suggested history of *S. mansoni* and *S. haematobium* infection and presence of infected family members as key risk factors of schistosomiasis (Dawaki *et al.*, 2016). This can be explained by sustained presence of the parasite in common water sources. Some studies have highlighted that it is actually the frequencies of water contact (taking baths, swimming, and wading in possibly infested streams) that is associated with higher schistosomiasis prevalence (Ismail *et al.*, 2014; Abdulkareem *et al.*, 2018). Other risk factors identified in literature include lack of portable drinking water, unemployment and lack of knowledge about schistosomiasis (Abdulkareem *et al.*, 2018).

The insignificant variation ($p=0.735$) between boys (16.7%) and girls (17.5%) could be as a result of similar water contact patterns between boys and girls despite disparities in recreational activities like swimming and chores like fetching water from and laundering in rivers characterizing most of the Zimbabwean rural societies. The findings also dispels myths that males have higher schistosomiasis prevalences relative to women (Bruun and Aagaard-Hansen, 2008). Instead women tend to have higher prevalence due to social and occupational roles. Social and occupational roles influence exposure patterns and perceptions of illness (haematuria is sometimes confused with menstrual periods and women are sometimes forced to ignore symptoms) often leaves females with the greater burden of schistosomiasis; knowledge of those disparities as well as exact prevalences in both genders is key in coming up with effective schistosomiasis control programmes (Bruun and Aagaard-Hansen, 2008). Differences in exposure, vulnerability, access to treatment and health outcomes that exist between men and women have been recognized in many communities. This is also explainable by the fact that most women and girls in many low to middle income communities with schistosomiasis are in the low socioeconomic class (Feasey *et al.*, 2010). Studies in Igbokuta Village, Nigeria reported higher prevalence in girls (61%) relative to boys (39%) (Oluwasogo and Fagbemi, 2013). Other studies such as the one done in Kaduna state, Nigeria have however highlighted higher prevalence in boys 14.8% than girls (5.4%) (Bishop and Akoh, 2018). This shows how schistosomiasis prevalence is sensitive to social and occupational activities (swimming, washing and irrigation farming) variations that occurs from region to region

depending on people's cultures and/or religions. In urban centres it has been highlighted that boys could be 7.8 times more likely to be infected with schistosomes than girls (M'Bra *et al.*, 2018). This is more likely due to the fact that young boys in urban centres are the only group that have outdoor activities that may lead contact with water such as swimming in ponds and other open water bodies.

Agricultural and fishing activities as well as lack of basic infrastructure in many communities are among key factors bringing people in contact with possibly contaminated water (Yang *et al.*, 2009; Mewabo *et al.*, 2017; Abdulkareem *et al.*, 2018; Hajissa *et al.*, 2018); often school going children are central in many agricultural activities in rural Zimbabwe. Kabuyaya *et al*, (2017) reported that school going children from households headed by women were at a higher risk to be infected by *S. haematobium* relative to those with both parents. Women headed households often rely more on children for regular chores like washing and fetching water (Kabuyaya *et al.*, 2017). Poor people often have poor access to safe water and sanitation as well as low levels of knowledge. Thus schistosomiasis prevalence is generally highest amongst the poorest regions in endemic countries (Bruun and Aagaard-Hansen, 2008). A study done in northern Côte d'Ivoire however contradicted the link between socioeconomic status and schistosomiasis prevalence (M'Bra *et al.*, 2018), further highlighting the complexities in schistosomiasis epidemiology. Thus there is a continuing need for more immuno-epidemiological studies as strategies to eliminate schistosomiasis are both being formulated and implemented (for monitoring and evaluation).

The study has demonstrated that age is a factor; the older the child the higher the prevalence (10.5%, 24.0% and 30.7% for 1-5, 6-10 and 11-15 years respectively). Some studies have also have also highlighted similar findings. Children between 10 and 15 years of age are 3.8 times more likely to be infected than their younger counterparts aged 5-10 years, who are in turn more likely to be infected than pre-school aged children (M'Bra *et al.*, 2018). Children 5 years and under typically have very low to nil prevalence depending on region and social fabrics; they are often too young to engage in many regular activities that brings them in contact with schistosome infested waters (Bishop and Akoh, 2018).

A long history (1967 to 2001) of focal molluscicide using niclosamide as well as systematic screening and treatment of all residents in the Zimbabwean side of Kariba dam has been used to explain lower prevalence observed in this region when compared to the Zambian side where no such programs exist (Chimbari, 2012). Many other examples in Zimbabwe, such as the

Hippo Valley Sugar Estates and Mushandike schistosomiasis control programmes, also highlight that sustained multifaceted control programmes significantly lead to reduction in prevalence and morbidity (Chimbari, 2012). The World Health Assembly in May of 2012 announced the feasibility of schistosomiasis eradication and encouraged water, sanitation and hygiene education (WASH) as components of an integrated control and elimination strategy (Grimes *et al.*, 2015). Praziquantel mass drug administrations and WASH programmes (Table 4.5) help in reducing environmental contamination with schistosome eggs as well as people coming in contact with contaminated water bodies. Washing with soap also helps as soap is known to be toxic to cercariae, miracidia and some freshwater snails (Grimes *et al.*, 2015).

Table 4.3: WASH components key in schistosome transmission control (Grimes *et al.*, 2015)

Domain	**Key points**
Water	• Safe water supplies help in reducing exposure. • Infrastructure such as sinks and showers limit contaminated water contact.
Sanitation	• Eggs in latrine pits or proper sewer lines cannot sustain schistosome transmission.
Hygiene	• Soap is toxic to miracidia, cercariae and intermediate host snails

Mass drug administration of praziquantel, WASH, health education and community mobilizations are imperative in the fight against schistosomiasis (Dawaki *et al.*, 2016). Control of the intermediate host snails (*Bulinus globosus* and *Biomphalaria pfeifferi*) is also central to enhance the effectiveness of the treatment based approach (community praziquantel mass drug administrations) (Chimbari, 2012). Studies also suggested that for effectiveness, educating communities about schistosomiasis should be an integral part of all mass drug administration campaigns (Chisango *et al.*, 2019). Continuous evaluation of all possible risk factors is essential if schistosomiasis is going to be eliminated from endemic communities or if a significant positive impact is to be made in its control.

4.5. Conclusion

Age and location are important factors in determining schistosomiasis morbidity or prevalence. Prevalence in rural endemic areas is similar between boys and girls.

4.6. Recommendations

- There is need for regular epidemiological surveys to both inform policies and strategies in combating schistosomiasis as well as guide monitoring and evaluation of implemented programmes.
- Regular assessment of all possible risk factors including those that could be dynamic and/or area specific.

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CHAPTER 5

SYNTHESIS OF RESEARCH FINDINGS AND CONCLUSIONS

5.1. Synthesis of research findings

This thesis reports on the project that was conducted to answer the overall objective of evaluating frequencies of genetic polymorphisms in TNF-α and IL-10 cytokine gene promoter regions in Zimbabwe. The regions are known to influence serum cytokine balance that is key in determining the morbidity and pathological consequences in many helminthic infections such as *S. haematobium* infections. The proinflammatory cytokine (TNF-α) and anti-inflammatory (IL-10) are thought to determine susceptibility to *S. haematobium*. The study was also aimed at making inferences on the potential impact of those single nucleotide polymorphisms (SNPs) on immunological profiles and drug administration outcomes. The studies presented in this thesis showed that the overall prevalence of urogenital schistosomiasis among the children surveyed was 17.1% and gender specific prevalence was indistinguishable statistically (17.5% in girls and 16.7% in boys; $p = 0.735$). Age group specific findings highlighted significant difference in prevalence between primary school children (26.8%) and preschool children (10.5%). Praziquantel treatment reduced prevalence among the study participants as reinfections were only recorded in few children (6/59; 10.2% of previously infected children). Praziquantel treatment has been demonstrated to markedly alter polarization of schistosome-specific cytokine responses, and those changes, particularly in response to egg-stage parasites, are thought to promote resistance to reinfection (Bourke *et al.*, 2013)**.** Thus the noted significant decline in prevalence and very low rates of reinfection following praziquantel treatment could be attributed to the said effects. There is however need to correct for other known effective interventions like health information about *S. haematobium* given to children and guardians.

Allele or genotype frequencies and the resultant cytokine levels were as expected among African children; with the TNF-α-308 GA and IL-10-819 CT genotypes having the highest frequency. The TNF-α AA, GA and GG genotypes were associated with high, moderate to high and low production of TNF-α, respectively. This is consistent with a previous study showing bi-allelic single nucleotide substitution of G (TNFA1 allele) with A (TNFA2 allele) polymorphism at −308 nucleotides upstream from the transcription initiation site in the TNF-α promoter is associated with elevated TNF-α levels (Elahi *et al.*, 2009). The IL-10 TT genotype corresponded with significantly lower IL-10 levels when compared with CC (which

had highest levels) and CT genotypes. The impact of SNPs in IL-10 promoter region on circulating levels of the cytokine is also consistent with findings from other studies (Makwikwi and Mduluza, 2019). TNF-α -308 G/A and IL-10 -819 C/T polymorphisms studied here were not associated with susceptibility to *S. haematobium* infection as the distribution of genotype frequencies did not differ significantly between the infected and uninfected participants. Higher TNF-α and lower IL-10 levels were negatively associated with schistosomiasis. Although cause and effect could not be determined, previous reports that high levels of IL-10 predispose to reinfection (Baran *et al.*, 2008; Sun *et al.*, 2010; Santos *et al.*, 2017) our observation that no individuals with the IL-10 TT genotype (which was also associated with lower IL-10 levels) were infected, support that lower levels of IL-10 confer protection against schistosomiasis infection. Despite the association between cytokine levels and schistosomiasis and the expected association between the various genotypes and cytokine levels, the genotypes did not individually significantly associate with susceptibility to infection. The findings therefore further highlight the complexities in immunity and host-parasite interactions. Nevertheless, since the genotypes are independent variables, chances of matching exact genotypes that will have low TNF-α and high IL-10, hence conferring increased susceptibility to *S. haematobium* infection, are low. In addition the impact of individual genotypes will probably be more marked if corrections for other known risk factors were instituted.

As highlighted above this present study has established that high serum levels of IL-10 and low TNF-α as positively linked with *S. haematobium* infection, and while cause and effect in this relationship was not proved in this study, it can be inferred from observations in the present study as well as previous studies. IL-10 is a potent anti-inflammatory cytokine produced by lymphocytes (Njaanake *et al.*, 2014). One study reported IL-10 elevation in chronic helminth infection (Arinola *et al.*, 2015) thus the susceptibility noted could potentially be a result of chronic infection and not as a result of IL-10 being a risk factor, however other studies have reported no elevation of IL-10 after *S. haematobium* infection (Lo *et al.*, 2018). Furthermore, other studies with IL-10 being reported to be a risk factor of *S. haematobium* infection/reinfection after adjusting for other variables (Van Den Biggelaar *et al.*, 2002; Bustinduy *et al.*, 2015; Elfaki *et al.*, 2016). These studies, together with our observation that no individuals with the IL-10 TT genotype (corresponding with low IL-10 levels) were infected support that low IL-10 is protective against infection and that high IL-10 is a risk factor for infection. Single nucleotide polymorphism in the promoter regions of IL-10 have also been associated with many disease such as psoriasis, gastric cancer and dengue (Baran *et al.*, 2008;

Sun *et al.*, 2010; Santos *et al.*, 2017). This study has also linked higher TNF-α serum levels to protection against *S. haematobium* infection. Most studies however have failed to establish any link between TNF-α serum levels and *S. haematobium* infection but rather to serious pathological developments in chronic infection (Bustinduy *et al.*, 2015). TNF-α has been implicated in the pathogenesis of many diseases and in influencing disease susceptibilities (Elahi *et al.*, 2009). Some studies detected elevated levels of TNF-α in infected individuals relative to uninfected controls especially in acute schistosomiasis (Coutinho et al. 2006). The findings of this study however contradicts those observations as low levels of TNF-α were associated with infection, which actually support the inferences that higher levels TNF-α are actually protective against *S. haematobium*.

Praziquantel has a significant role in the elimination of schistosomiasis or at least in significant reduction of schistosome burden with in endemic communities. The MDA that associated with the study led to fewer and fewer reinfections. Significant reduction in prevalence a year after treatment with praziquantel was also reported by a study done in central Sudan, highlighting the central role praziquantel has in any schistosomiasis elimination programme (Ahmed *et al.*, 2012). Similar findings were reported as well by Chisango *et al* (2019); in their study praziquantel did not only led to significant reduction of prevalence but infection intensities as well (Chisango *et al.*, 2019). This was based on egg reduction rates cure rates after single or divided 40mg/kg dose(s) of praziquantel. The egg reduction rates ranged between 80 to 100% (Taylor, 2008; Ojurongbe *et al.*, 2014; Chisango *et al.*, 2019). As highlighted above the effectiveness of this significant reduction in prevalence after treatment could also be attributed to the knowledge and/or information about schistosomiasis given to the children and guardians on every encounter. The few reinfections however points to the fact that praziquantel cannot be used alone but for significant and sustained effectiveness it should be combined with other known effective strategies such as WASH (water, sanitation and hygiene), snail control and health education. Mduluza *et al*. (2001) reported significant reduction in prevalence and infection intensities of *S. haematobium* when praziquantel was given every 8 months for 2 years in children under 6 years living in endemic areas. They also noted a shift in antibody profiles towards protective antibodies (IgA and IgG1) further highlighting the impact of praziquantel in schistosomiasis eradication (Mduluza *et al.*, 2001; Chisango *et al.*, 2019).

Mass drug administration (MDA) programs that include pre-school and school going children as well as adults have been found to be cost-effective based on the incremental cost effectiveness ratio (ICER) per disability-adjusted life-year (DALY) averted (ICER $167 per

DALY averted), in a study done in Côte d'Ivoire (Lo *et al.*, 2015). Repeated school based MDA programmes have many incremental and additional (externalities) benefits such as Quality-Adjusted Life-Years (QALYs) gained, rapid reduction of prevalence and infection intensity, increased coverage and infection interruption despite seemingly increasing costs (King *et al.*, 2011). Externalities include enhanced HIV and other STI prevention strategies; depending on HIV prevalence in any given region, treatment of schistosomiasis has been linked to decreased transmission of HIV (Ndeffo Mbah, Gilbert and Galvani, 2014). Thus sustained and frequent community-wide MDA programmes, multi-sectorial approaches and community participation, provision of clean water, sanitation and health education are recommended even in communities with low schistosomiasis prevalence for elimination and eventual eradication (Mbah *et al.*, 2013; Lo *et al.*, 2015; Tambo *et al.*, 2017; Ndassi *et al.*, 2019). There are many personal and community factors that affect praziquantel coverage in any MDA programme such as socio-political set-ups with in communities, culture, religious beliefs, knowledge, age groups, mode used (central or door-door distributions), gender, comorbidities, distance from distribution centres and availability of supervision (Dabo *et al.*, 2013). Ill people tend to seek medical attention more readily and some religious sects do not allow members to seek modern medical help. If school based distribution modes are used certain clusters wont access praziquantel especially in most sub-Saharan countries with many communities with poor school enrolments/coverage (Turner *et al.*, 2017). In a study done in Nigeria; community based delivery was found to be the best mode of distributing praziquantel as it achieved 72.25% coverage compared to school (44.30%) or health facility (28.50%) based modes (Mafe *et al.*, 2005). Direct observation, as might happen when medical supervision is available, increases compliance. Typically participation in mass drug programmes is better with males, school going children and those who have participated before (Tallo *et al.*, 2008).

Many variable risk factors have been associated with *S. haematobium* infection such as age group, location or area of residence, education level, occupation, source of water, gender, frequency of stream activities and socioeconomic class (Ndassi *et al.*, 2019). The findings of this study have highlighted age and location as important factors in determining schistosomiasis prevalence. The study has also established that prevalence in rural and farming communities within endemic areas is similar between boys and girls. The lack of significance differences in infection levels between boys and girls is likely because most water-related chores and recreational activities are similar and often overlap within the age groups studied in many rural communities. Thus similar prevalences are expected in boys and girls; differences

are expected in adults and gender role differences become more marked. Thus elimination and eradication programmes should not discriminate based on gender. The World Health Organization's set goals of controlling morbidity by 2020 and eliminating schistosomiasis as a public health problem by 2025 in many communities is achievable only if as highlighted above all inclusive approaches are applied especially in moderate to high prevalence regions like most of sub-Saharan Africa which house 85% of those infected (Taylor, 2008; French *et al.*, 2018; Toor *et al.*, 2018). Programmes such as sustained praziquantel treatments or chemoprophylaxis; development of more sensitive diagnostic tools; potential vaccine candidate development; enhanced snail control; WASH and health education can lead to elimination and eradication of schistosomiasis (Inobaya *et al.*, 2014; Bergquist *et al.*, 2017; Secor and Colley, 2018; Toor *et al.*, 2018). There is still need for more financial and human resources as well as significant sustained political will if significant impact is to be made in the fight against schistosomiasis in Africa (French *et al.*, 2018). It is also important to stress that risk factors for schistosomiasis are often variable from community to community hence the need for research supported community specific guidelines rather than generic approaches for all if elimination of the infection is to be realized in this generation (Bergquist *et al.*, 2017).

5.2. General limitations

Some participants were not willing to provide blood for genotyping experiments. Of those willing (or willing guardians) some were unable to provide adequate blood for both genotyping and cytokine plasma level assessments especially in the pre-school age group. Resources and time limited the number of cytokines to be investigated as well as number of children to recruit. The limitations stated does not invalidate the important conclusions drawn from the study as the cytokines investigated are representative and the children numbers were well above calculated sample sizes.

5.3. Overall conclusions

Prevalence of *S. haematobium* in endemic rural and farming communities of Zimbabwe is similar between boys and girls. The overall prevalence of schistosomiasis observed (17.1%) puts the regions within the moderate range (10 – 49% prevalence). Age and location are important risk factors for schistosomiasis in children living in endemic regions. The older the child the higher the risk of getting infected by *S. haematobium* (10.5% in 0-5; 24.0% in 6-10 and 30.7% in 11-15; $p = 0.000$). IL-10 -1082 G/A, IL-10 -819 C/T and TNF-α -308 G/A single nucleotide polymorphisms are not significantly associated with susceptibility to *S.*

haematobium infection. TNF-α genotypes AA, GA and GG are associated with high, moderate to high and low production of TNF-α respectively. IL-10 TT, CT and CC genotypes are associated low, moderate and high IL-10 plasma levels respectively. Higher TNF-α and lower IL-10 serum levels are protective against schistosomiasis.

Cytokine production is subject to many regulatory factors and the resultant levels circulating are a direct result of those opposing factors. Thus genotypic association with disease susceptibility and severity is a complex interplay of factors, where SNPs are one factor. A critical balance between TNFα and IL-10 is essential in determining susceptibility, morbidity and immunity to *S. haematobium*. Low IL-10 and high TNF-α leads to severe morbidity and/or severe immunopathological condition. Low TNF-α and/or high IL-10 leads to susceptibility, low immunity and higher chances of chronic infection. Thus an optimal balance of TNF-α and IL-10 levels is required for one to be immune and also protected against severe pathology in case one is infected of *S. haematobium*. Praziquantel treatment reduces infection burdens among children.

5.4. Implications and recommendations

- Inclusion of all age groups in non-gender specific prevention, management and control programmes in children will yield more positive results.
- Future studies which include many cytokines and all possible single nucleotide polymorphisms known to influence cytokine gene expression will give a better picture on susceptibility and immunity to schistosomiasis and/or other helminthic infections.
- Regular prevalence and infection intensity studies to help monitor and evaluate effectiveness of mass drug administration activities and well as checking age groups at high risk who might have been excluded.
- Regular risk factor assessments as this will help in shaping control and elimination programmes.
- Zimbabwe and indeed other nations should learn from past experiences and avoid overreliance on treatment/curative approach, but rather combine this with snail control and WASH programmes.
- Other risk factor corrected genotyping and plasma cytokine level assessments could also help determine the existence or inexistence of association between certain genotypes (of TNF-α, IL-10 and/or other cytokines) and susceptibility to *S. haematobium* infections.

- Other cytokine and T-cell stimulation studies may provide more conclusive linkages between infection and cytokine responses in settings that are endemic for multiple parasites and multiple co-infections.

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Appendix 1: Abstract 2018 Bryden Country School bulletin

Neglected Tropical Diseases (NTDs): Schistosomiasis and Soil Transmitted Helminths

Based on World Health Organization (WHO) classification NTDs are a diverse group of about seventeen communicable diseases afflicting around 149 mostly resource poor countries in sub-tropical and/or tropical regions of the world. They affect more than a billion people and cost billions each year. With wide ranging local and global public health initiatives WHO is looking to eradicate all in the near future starting with at least two by 2020. NTDs specified by WHO include dengue, buruli ulcer, leishmaniasis, hydatidosis, soil transmitted helminths, trachoma, lymphatic filariasis, onchocerciasis, leprosy, Chagas disease, trypasomiasis and schistosomiasis. Zimbabwe is plagued mostly by schistosomiasis/bilharzia and soil-transmitted helminths (incl. intestinal worms). Network of rivers and other water bodies in Zimbabwe and socioeconomic challenges are promoting transmission of these NTDs. Co-morbidity of these diseases is common particularly in school going children. Falciparum malaria and HIV/AIDS are also common in those co-morbidity situations. Their obvious consequences include chronic anaemia, higher chances of contracting sexually transmitted diseases incl. HIV (esp. with schistosomiasis), poor prognosis and intervention outcomes especially in co-infection with HIV/malaria, poor child development and performance outcomes even in later life, low fertility and in some cases death. Adults and pets often carry the infectious organisms of NTDs without obvious signs and symptoms but regularly causing re-infections in children. Effective vaccines are still elusive and far into the future. Repeated mass drug administrations in children with chemotherapeutic agents; praziquantel (schistosomiasis) and albendazole (soil transmitted helminths) are the mainstay in control efforts of these NTDs. Drug resistance, poor compliance, side effects, religious beliefs and other misguided myths, unavailability and costs are hampering successes of those medicines. These efforts should also include regular deworming of pets and adults. Water, sanitation and hygiene (WASH) is key in prevention and pathogen life cycle disruptions. Since health education is key in prevention, control and management; Schools have a bigger role to play in the envisaged eradication of NTDs.

Prepared by Amos Marume (+263772687090 or stafirejee@gmail.com)

Sponsor:

Main branch

60 G. Silundika Ave/5th St, Harare, Zimbabwe - +2634792076 or guardianpzim@gmail.com

Other branches

K215 Katanga, Norton, Zimbabwe - +263622591 or guardiankatanga@gmail.com

65 Rezende/R. Mugabe, Harare, Zimbabwe - +2634770261 or guardianrezende@gmail.com

Appendix 2: Abstract of published book chapter

Chapter 6

Global Control Efforts of Schistosomiasis and Soil-Transmitted Helminthiasis

Takafira Mduluza, Tawanda J. Chisango,
Agness F. Nhidza and Amos Marume

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/65282

Abstract

Schistosomiasis is a waterborne disease whose life cycle involves freshwater sources conducive for the survival and reproduction of aquatic snails that form a connective link between man and water in the life cycle and transmission of schistosomiasis. The African region has network of rivers with freshwater suggesting the presence of schistosomiasis and difficulty to control. Some communities, due to socioeconomic challenges, have inadequate sanitation and water supply; use of bush toilets for excretion is commonly practiced. These conditions in Africa also promote transmission of soil-transmitted helminthiasis. The World Health Organization (WHO), in response to the public health and socioeconomic impact of neglected tropical diseases, is coordinating strategies for the control and elimination of the diseases including schistosomiasis and soil-transmitted helminthiasis. As one of the milestones, mapping of neglected tropical diseases in the African region has been prioritized for the implementation of control strategies. In countries where mapping has been completed, WHO and its partners are supplying medicines required for annual mass treatment for preventive chemotherapy and encourage countries to take ownership in implementing complementary strategies for morbidity control, elimination and eradication of country-specific neglected tropical diseases. The mainstay of helminthiasis control is preventive chemotherapy, targeting school age children to prevent morbidity and development of pathological manifestations, including urogenital schistosomiasis that is understood to contribute to HIV transmission. Vaccines are still to be discovered and designed, with many possible antigen candidates, but however the immune responses are still to be fully understood. There is need to understand the subtle link between each component of the immune responses and the host immunogenetics impacting on the translated immunological response of cytokines that are delicately controlled for cellular immunity and antibody production. Currently, preventive chemotherapy treatment is the only

Appendix 3: Ethical clearance letter

Infectious Diseases Immunology
Professor Takafira Mduluza
BApplSc. Biol. & Biochem (UZ); MPhil. (UZ); PhD (Glasgow-UK)
BIOCHEMISTRY DEPARTMENT

P.O Box MP 167
Mount Pleasant
Harare, Zimbabwe
Telephone: 303211 ext. 17134
Cell: +263 77 363 3682
Telex: 26580 UNIVZ ZW
Telegrams: UNIVERSITY

UNIVERSITY OF ZIMBABWE

21st February 2017

To whom it may concern

RE: CONFIRMATION OF ETHICAL CLEARANCE

Student Name: Amos Marume

Student Reg. No.: 213524974

Programme: PhD Medical Microbiology

This serves to confirm that the research activities which culminated into the thesis titled "**ROLES OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE PROMOTER REGIONS OF TUMOR NECROSIS FACTOR-α AND INTERLEUKIN-1O GENES IN *SCHISTOSOMA HAEMATOBIUM* INFECTION SUSCEPTIBILITY**" were approved as part of broader main proposals with the following ethical clearance details;

- MRCZ/A/1710 (Medical Research Council of Zimbabwe)
- BE467/16 (Biomedical Research Ethics Committee – UKZN)

Should there be need for more information please contact the undersigned.

Yours Sincerely

[redacted]

T. Mduluza Ph.D.
Professor: Biochemistry & Immunology
Honorary Professor School of Laboratory Medicine & Medical Science, UKZN

Professor T. Mduluza
mduluza@medic.uz.ac.zw or tmduluza@yahoo.com

Appendix 4: MRCZ approval letter

Telephone: 791792/791193
Telefax: (263) - 4 - 790715
E-mail: mrcz@mrcz.org.zw
Website: http://www.mrcz.org.zw

Medical Research Council of Zimbabwe
Josiah Tongogara / Mazoe Street
P. O. Box CY 573
Causeway
Harare

CONTINUING APPROVAL LETTER

REF: MRCZ/A/1710 **12 November 2017**

Prof T Mduluza
UZ College of Health Sciences
P.O Box MP167
Mount Pleasant
Harare

RE:- Monitoring The Effects of Mass Drug Administration of Praziquantel During A National Schistosome Control Programme on The Prevalence Of Autoimmune Reactivity And Allergic Sensitization.

Thank you for the application for review of Research Activity that you submitted to the Medical Research Council of Zimbabwe (MRCZ). Please be advised that the Medical Research Council of Zimbabwe has **reviewed**and **approved**your application to continueconducting the above titled study.

Thisapproval is based on the review and approval of the following documents that were submitted to MRCZ for review:-

a) Completed MRCZ Form 102

- **APPROVAL NUMBER : MRCZ/A/1710**

This number should be used on all correspondence, consent forms and documents as appropriate.

- **TYPE OF MEETING : Full Board**
- **EFFECTIVE APPROVAL DATE : 12 November 2017**
- **EXPIRATION DATE : 11 November 2018**

After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the MRCZ Offices should be submitted three months before the expiration date for continuing review.

- **SERIOUS ADVERSE EVENT REPORTING**: All serious problems having to do with subject safety must be reported to the Institutional Ethical Review Committee (IERC) as well as the MRCZ within 3 working days using standard forms obtainable from the MRCZ Offices or website.
- **MODIFICATIONS**: Prior MRCZ and IERC approval using standard forms obtainable from the MRCZ Offices is required before implementing any changes in the Protocol (including changes in the consent documents).
- **TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the MRCZ using standard forms obtainable from the MRCZ Offices or website.
- **QUESTIONS**: Please contact the MRCZ on Telephone No. (04) 791792, 791193 or by e-mail on mrcz@mrcz.org.zw

Other

- Please be reminded to send in copies of your research results for our records as well as for Health Research Database.
- You're also encouraged to submit electronic copies of your publications in peer-reviewed journals that may emanate from this study.

YoursFaithfully

[redacted]

MRCZ SECRETARIAT
FOR CHAIRPERSON
MEDICAL RESEARCH COUNCIL OF ZIMBABWE

MEDICAL RESEARCH COUNCIL OF ZIMBABWE
2017 -11- 12
APPROVED
P.O. BOX CY 573 CAUSEWAY, HARARE

PROMOTING THE ETHICAL CONDUCT OF HEALTH RESEARCH

Appendix 5: Specimen storage informed consent form

University of Zimbabwe

UNIVERSITY OF ZIMBABWE

SPECIMEN STORAGE INFORMED CONSENT FORM

STUDY TITLE:

ROLES OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE PROMOTOR REGIONS OF TUMOR NECROSIS FACTOR-α AND INTERLEUKINE-1O GENES IN *SCHISTOSOMA HAEMATOBIUM* INFECTION SUSCEPTIBILITY

Protocol Version: Ver1. 01.11.2012

SPECIMEN STORAGE

Consent Version

(English)

PRINCIPAL INVESTIGATOR: Mr A. Marume

PHONE: 0772687090

INTRODUCTION:

You have decided to take part in the investigational research study named above, sponsored by the University of Zimbabwe and Ministry of Health & Chid Welfare. While your child is in this study, blood 2.5 – 5 mls will be collected from your child. You are kindly being asked to agree to the storage of these samples for use during the study and after the study has ended. We may also ship these samples to another laboratory outside Zimbabwe if the need comes. This consent form gives you information about the collection, storage, and use of these

samples. These samples will be useful in the first next 5 years the Mass Drug Administration of praziquantel research programme is running. The study staff will talk to you about this information. Please ask if you have any questions. You will be asked to sign or make your mark on this form to indicate whether you agree to have your child's samples stored and tested. You will be offered a copy of this form to keep.

YOUR PARTICIPATION IS VOLUNTARY:

Allowing your samples to be stored is completely voluntary. You may decide not to have any samples stored other than what is needed to complete this study and still be in this research study or any future study.

Even if you decide now that your samples can be stored for the duration of the research, you may change your mind at any time. If this happens, you must tell the study staff that you have changed your mind. If you decide not to have your samples stored or used for future research, they will be destroyed at the end of the study.

PURPOSE:

The specific research to be done on your blood samples will be to analyse for markers of allergic reactions after treatment. The samples will only be used to look for antibodies as markers of reaction and effect of treatment on the infections, damage caused by infection, or how your body reacts to the infection. For example, the tests may look at cells, proteins, and other chemicals in your body. Tests may also examine your genes (DNA), since they might affect your response to parasitic infection in important ways. For example, your genes may make you more or less susceptible to becoming infected, your responses to infection or to treatment stronger or weaker, or make development of allergic reactions progress faster or slower. No other kinds of genetic test will be done by anyone on your stored specimens without first explaining the test to you and obtaining your permission.

The study researchers do not plan to contact you or your regular doctor with any results from tests done on your stored samples. This is because research tests are often done using experimental procedures, so the results may not help for making decisions on managing your health. In the very rare case that a specific test result gives important information about your health, the researchers will contact you. If you wish to be contacted with this type of test result, you must give the study staff any change to your contact information. If you have a regular

doctor and you want the study staff to tell this doctor your child's test results, you must give the study staff your doctor's contact information.

Your samples will not be sold or used directly to produce commercial products.

Research studies using your samples will be reviewed by the Medical Research Council of Zimbabwe.

PROCEDURES:

Each time your child's blood is drawn, up to 5 mL (which is about 1.5 teaspoons) of the sample may be stored. For each sample of blood, part of the sample will be tested immediately and the rest will be stored.

Your blood will be stored safely and securely in a storage facility at the University of Zimbabwe-Biochemistry Department. Only the people who work at the facility and approved researchers will have access to your samples. The people who work at the facility will not have any information that identifies you. The approved researchers may be given information about you such as your age and sex, but they will not be given your name or any other information that identifies you. Your samples may be shipped to approved researchers who work outside of Zimbabwe, if the need arises for some specialized assays not available in Zimbabwe. There is no time limit on how long your samples will be stored but we anticipate during duration of the MDA.

RISKS and/or DISCOMFORTS:

There are few risks related to storing your samples. When tests are done on the stored samples there is a rare but possible risk to your privacy. It is possible that if others found out information about you that is learned from tests (such as information about your genes) it could cause you problems with your family (having a family member learn about a disease that may be passed on in families).

POTENTIAL BENEFITS:

There are no direct benefits to you from having your child's samples stored. You and others could benefit in the future from research done on these samples.

CONFIDENTIALITY:

To keep your information private, your samples will be labelled with a code that can only be traced back to your study clinic. Your name, where you live, and other personal information will be protected by the study clinic. When researchers are given your stored samples, they will not be given your personal information. Every effort will be made to keep your personal information confidential.

Efforts will be made to keep your study records and test results confidential. You will be identified by a code, and personal information from your records will not be released without your written permission. Any publication of this study will not use your name or identify you personally.

PROBLEMS OR QUESTIONS:

For questions about the storage of your samples, contact:

- The PI: Amos Marume

Paraclinical Department,

University of Zimbabwe

Churchill/University Ave

P.O. Box MP 167

Mount Pleasant, Harare

Ph: 0772687090

For questions about your rights as a research subject, contact:

- ***Medical Research Council of Zimbabwe***

National Institute of Health Research

Cnr Mazoe Street/ Josiah Tongogara Avenue

Harare

Ph :+263 4 791792, 791193

Cell : +263 772 433 166

SIGNATURE PAGE

CONSENT FOR SPECIMEN STORAGE

Please carefully read the statements below (or have them read to you) and think about your choice. No matter what you decide it will not affect whether you can be in the research study, or your routine health care.

_____ I agree to have samples of my child's blood sample, stored and used for future testing related to allergy & autoimmunity.

_____ I agree to have samples of my child's blood sample, stored and used for future testing related to allergy and autoimmunity. However, I do not agree to have genetic testing performed on my samples.

_____ I do not agree to have samples of my child's blood, stored and used for future testing related to allergy and autoimmunity.

_____

Name of Child (please print)

_____ _____

Parent's/Guardian's Name (print) Signature or Mark and Date

_____ _____

Study Staff Conducting Study Staff Signature and Date

Consent Discussion (print)

_____ _____

Witness Name (print) Witness Signature and Date

(As appropriate)

Appendix 6: Informed consent form for parental consent (Shona version)

UNIVERSITY OF ZIMBABWE

MRCZ/A/1958

BVUMIDZO YEVABEREKI

MUSORO WECHIRONGWA

KUONGORORWA KEROPA REVANA VARIKURAPWA CHIRWERE CHECHIPFUNGA

Mutungamiri wechirongwa: **Mr Amos Marume** (MSc)-0772687090

Vatevedzi : **Professor Takafira Mduluza**, [PhD]- 0773633682

Dr J. Mann [PhD]

Zvamunofanira kuziva nezvechirongwa ichi:

- Tinokupai chibvumirano ichi kuti muverenge pamusoro pechinangwa, zvingangonetsa, uye nezvingangobatsira muongororo iyi.
- Zvirongwa zvinoitwa zvinozobva pane ruzivo rutsva rwakanakisa rwatinenge tavanarwo
- Chinangwa chikuru cheongororo ndechekuwana ruzivo rutsva runozobatsira pane ramangwana.
- Hatingavimbisi kuti ongororo iyi ichakubatsirai.
- Munekodzero yekuramba kupinda/ kuve , kana yokubvuma kupinda izvozvi, uye yekushandura pfungwa dzenyu pamberi apo.
- Chero sarudzo yamaita, hazvikanganisi mabatirwo enyu enguva dzese

- Ndapota nyatsodzverai chibvumirano chino nemazvo. Bvunzai mibvunzo musati maita sarudzo- hamumanikidzwi kupinda.

Chinangwa cheongororo

Muri kukumbirwa mvumo yekuti mwana wenyu apinde muongororo iyi nokuti muri mubereki/ munhu mukuru anogara mudunhu rino. Muongororo/ mutsvakiridzo iyi tiri kuedza kuona zvinoitika kana munhu anwa mapiritsi anorapa bharaziya . Vana vari muzvikoro ndivo vachange vachipihwa mapiritsi aya, zvichireva izvo vanogona kubuda munyawiri muviri wese. Isu vechirongwa chino tinoda kuongorora zvinokonzeresa munyawiri uyu.

Mafambiro echirongwa

Kana mabvumidza mwana kupinda muongororo/tsvakirodzo iyi muchange murimumwe wechikwata chevanhu vari munzvimbo dzakasiyasiyana vachange vachimirira vamwe vavo nezveongororo iyi yekuti vamwe vanobuda munyawiri asika vamwe hapanachinoitika. Tichabvunzazve vana vechikoro ava kuti tigoziva nzvimbo dzavanopinda mumvura. Izvi zvinotibatsira kuti tigoongorora kunobva chirwere ichi chebharazuya.

Zvingangonetsa

Hapana zvinganetsa pakupinda muongororo iyi. Hurukuro ingangotora maminitsi mashanu kanasvika gumi enguva yavena. Tichatorazve ropa ringanhoita sipunu rimwe kana maviri ekuti tizoshandisa kuongorora zviratidzwa zvwnyawiri urwu.

Zvamunotarisirwa kuwana

Hapana chatingati muchawana semuripo nekuva kwenyu muongororo/tsvakiridzo iyi. Muchawana ruzivo rutsva runogona kukubatsirai kuchengetengetedza mhuri yenyu iri mutano. Zvekare, pfungwa dzenyu nezvamakasanganirana nazvo zvingangozobatsira kuti kuve nezvirongwa zvirinani zveutsana/kushambidzika munharaunda yenyu kana kune dzimwe nzvimbo dzenyika.

Muripo

Hamubadharwe kuva muongororo iyi/ hapana muripo nokuva mutsvangiridzo iyi.

Zvichabuda zvitsva muongororo

Tichagoverana nemi zvichawanikwa muongororo ino uye zimwe zvitsva zvatinenge tawana pamusoro pe tsvakiridzo iyi [yebharaziya nekurapwa kwayo nekubuda munyawiri].

Kuchengetedzwa kwezvinyorwa

Tichanyora pasi zita nekero yenyu, nozvimwewo zviri maererano nemi pamapepa. Hapana mumwe munhu anogona kuona zvinyorwa izvi kunze kwevari muongororo. Tichashandisa nhamba kwete zita, kuti vamwe vakuzivei. Magwaro ose ane zita nekero nezvimwe zviri maererano nemi zvamuno tiudza zvinochengetedzwa zvakakiyirwa, pane dzimwe nguva vaongorori vemitano yeongorori vangade kuona kuti ongororo iri kuitwa nemazvo here, ava ndivo vangazotarisa mapepa aya. Vanhu ava vanosanganisira veMedical Research Council of Zimbabwe kana kuti VeUniversity of Zimbabwe. Vese ava vanosungirwa kuchengetedza mazita enyu akavandika . kuchengetedzwa kwakasimbisiswa hatingakukomakedze nekuti mapepa emaresearch /ongororo haangadziviswi kana achidiwa navehurumende kana vematare emutemo. Tichaedza nepatinogona kuchengetedza zviri maererano nemi zvakawandika. Zvinobuda semhinduro yeongororo iyi kana zvinoshambadzirwa, hazvibudi zvine mazita enyu kana remwana kana zvimwe zvinoita kuti muzivikanwe kuti makanga muri mune chino chirongwa.

Kuda kwenyu

Mwana haamanikidzwi kupinda muongororo iyi. Kana masarudza kuti apinde muongororo iko zvino makasununguka kumuti abude muongororo iyi chero nguva.

Kana muine mibvunzo monogona kundibvunza ndakasununguka kuipindura. Munogonawo kubvunza vakuru vakuru [Professor Takafira Mduluza] veongororo vane mazita akanyorwa pazasi apa kana paine zvamusinganzwisisi maererano neongororo iyi ikozvino kana mave muongororo

Ndivanaani vandinofonera kana ndiine mubvunzo kana dambudziko

Kana muine mibvunzo maererano neongororo taurai navakuru veongororo vanonzi ma[principal investigator] [Amos Marume] panhamba dzinoti [0772687090]

Kana muine mibvunzo nezvekodzero dzenyu muchiongororo, ridzai rinhare kune veMedical Research Council of Zimbabwe

Josiah Tongogara/Mazoe Street

Causeway, Harare

Telephone [04 791 193/ 7907 15/791791]

Zvinorevei kunyora [kusaina] zita rako

Ini , nyakusaina pazasi, ndaudzwa chinangwa, mafambiro, zvingangonetsa nezvingangobatsira muongororo iyi. Ndapihwa rimwe reiri gwaro rekugara naro. Ndapihwa mukana wekubvunza mibvunzo chero nguva ipi zvayo. Ndazvisarudzira uye ndabvuma kuti mwana ave muongororo iyi. Isarudzo yangu kuti mwana abude muongororo, ndinoziva kuti hazvikanganise basa rangu kana chinzvimbo changu munharaunda. Ndinovimbisa kuti ndichatevedza zvandinenge ndakumbirwa kuita navashandi, navakuru vakuru veongororo iyi. Ndinovimbisa kuudza vashandi veongororo pakare pakarepo kana ini ndiine zvinetswa pandinenge ndiri muongororo.

Zuva ramurikusainira mwana kupinda muongororo, zvichireva zuva ranhasi, RINOFANIRWA kunge riri mukati memazuva akaratidzwa pachidhindo chiri papepa rimwe nerimwe. Mazuva akaratidzwa aya anotaridza kuti pepa rino riri kufambiswa munguva yakatenderwa, kureva kuti muchange muri muongororo iyi.

_____

Zita remwana (Nyorai nemavara makuru)

_____ _____

Zita remubereki (Nyorai nemavara makuru) Zuva

Zita kana chiratidzo chearikumirira mwana Zuva nenguva

Hukama nemwana arikupinda muchirongwa

Hwitinesi – pane avo vasingagoni Zita remumiririri arikutora bvumo ino kuverenga kana kunyora

Rimwe remagwaro echibvumirano ichi rakasainiwa rinofanirwa kuti 1] richengetedzwe mufaira nemukuru mukuru weongororo ,2] ripihwe kune uyo asarudza kupinda muongororo ne3] riiswe mufaira rekurapwa kweuyo asarudza kupinda muongororo.

Appendix 7: Informed consent form for parental consent

UNIVERSITY OF ZIMBABWE

MRCZ/A/1958

INFORMED CONSENT FORM

FOR PARENTAL CONSENT

PROJECT TITLE

ROLES OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE PROMOTOR REGIONS OF TUMOR NECROSIS FACTOR-α AND INTERLEUKINE-1O GENES IN *SCHISTOSOMA HAEMATOBIUM* INFECTION SUSCEPTIBILITY

Principal Investigator: **Mr A. Marume** (MSc)-0772687090

Co-Investigator(s): **Professor Takafira Mduluza**, [PhD]- 0773633682

Dr J. Mann [PhD]

ADD THE FOLLOWING PARAGRAPH TO ALL CONSENT FORMS

MORE THAN TWO (2) PAGES LONG (BEFORE ADDITION OF THIS PARAGRAPH)

What you should know about this research study:

- We give you this consent so that you may read about the purpose, risks, and benefits of this research study.

- Routine care is based upon the best known treatment and is provided with the main goal of helping the individual patient. The main goal of research studies is to gain knowledge that may help future patients.

- We cannot promise that this research will benefit your child. Just like regular care, this research can have side effects that can be serious or minor.

- You have the right to refuse to allow your child to take part, or agree for your child to take part now and change your mind later.

- Whatever you decide, it will not affect your child's regular care.

- Please review this consent form carefully. Ask any questions before you make a decision.

- Your choice to allow your child to participate is voluntary.

PURPOSE

You are being asked to allow your child to participate in a research study of **susceptibility to bilharzia and its treatment.** The purpose of the study is to assess if there is any **genetic association of cytokine gene polymorphism and susceptibility to schistosomiasis.** Your child was selected as a possible participant in this study because **this is the age that is commonly found infected with bilharzia and also their health and learning ability is greatly affected.** Also the children at this school will get the treatment from the currently bilharzia treatment underway throughout the country. We need to monitor their health status before and after the treatment is given. The information is important in our country for any likely development of allergy as caused by the worms when they die after treatment. We would like to get information for preparedness in the future activities of the mass treatment exercise by The Ministry of Health and Child Welfare. **This study will have a total of about 700 children recruited to represent others in the schools and we will have school children recruited from selected schools in other provinces.**

MRCZ/A/1958

PROCEDURES AND DURATION

If you decide to allow your child to participate, your child will be asked a few questions taking less than 5 minutes so that we know where they get in contact with water in rivers or streams and if there are things that cause allergy in their daily lives. Thereafter the child will be asked to provide urine and stool in small bottles so that we will be able to diagnose if they are infected with bilharzia and some worms. This process will be done over 3 consecutive days so that we don't miss any infection. A small blood sample 2,5 -5 mls will be taken from them and will used if they are infected with malaria and if there are markers that show any reaction to agents that cause allergy. The same procedure will be conducted before they receive treatment and 6 weeks after treatment to observe any changes to treatment and the development of allergic markers. We will monitor the children for any development of allergy throughout the exercise of bilharzia and worms treatment every year for the next 5 years.

RISKS AND DISCOMFORTS

The study does not involve any risks besides a temporary discomfort that may be experienced by child while taking venous blood due to the needle prick. However, this procedure is a common and normal as practised at most health centres and will be performed by qualified medical personnel from the Ministry of Health.

BENEFITS AND/OR COMPENSATION

Your child will benefit from this program by being one of the few children that will receive close monitoring and examination from the mass treatment of bilharzia and worm throughout the country. The study team cannot manage to examine closely everyone, the reason we have to select a few to represent others.

ALTERNATIVE PROCEDURES OR TREATMENTS

Praziquantel is the only drug available for treating bilharzia. Every child will receive this treatment for free. Treatment for the other worms would also be free.

MRCZ/A/1958

CONFIDENTIALITY

If you indicate your willingness for your child to participate in this study by signing this document, any information that is obtained in connection with this study that can be identified with your child will remain confidential and will be disclosed only with your consent, and when appropriate, your child's permission. Under some circumstances, the MRCZ and the local Institutional Review Board may need to review patient records for compliance audits.

ADDITIONAL COSTS

There are no costs involved with this study

IN THE EVENT OF INJURY

In the event of injury resulting from your child's participation in this study, treatment will be offered by the study.

VOLUNTARY PARTICIPATION

Participation in this study is voluntary. If you decide not to allow your child to participate in this study, your decision will not affect your or your child's future relations with research group and this institution, its personnel, and associated hospitals**.** If you decide to allow your child to participate, you and your child are free to withdraw your consent and assent and discontinue participation at any time without penalty.

ADDITIONAL ELEMENTS

None

MRCZ/A/1958

OFFER TO ANSWER QUESTIONS

Before you sign this form, please ask any questions on any aspect of this study that is unclear to you. You may take as much time as necessary to think it over.

AUTHORIZATION

YOU ARE MAKING A DECISION WHETHER OR NOT TO ALLOW YOUR CHILD TO PARTICIPATE IN THIS STUDY. YOUR SIGNATURE INDICATES THAT YOU HAVE READ AND UNDERSTOOD THE INFORMATION PROVIDED ABOVE, HAVE HAD ALL YOUR QUESTIONS ANSWERED, AND HAVE DECIDED TO ALLOW YOUR CHILD TO PARTICIPATE.

The date you sign this document to enrol your child in this study, that is, today's date, MUST fall between the dates indicated on the approval stamp affixed to each page. These dates indicate that this form is valid when you enrol your child in the study but do not reflect how

long your child may participate in the study. Each page of this Informed Consent Form is stamped to indicate the form's validity as approved by the MRCZ.

_____ _____

Name of Parent (please print) Date

_____ _____

Signature of Parent or legally authorized representative Time

_____

Relationship to the Participant

Signature of Witness Signature of Research Staff

(*Optional*)

YOU WILL BE GIVEN A COPY OF THIS CONSENT FORM TO KEEP.

If you have any questions concerning this study or consent form beyond those answered by the investigator, including questions about the research, your rights as a research Participant or research-related injuries; or if you feel that you have been treated unfairly and would like to talk to someone other than a member of the research team, please feel free to contact the Medical Research Council of Zimbabwe on telephone 791792 or 791193.

MRCZ/A/1958

Appendix 8: Manuscript accepted and proofreading done - Southern African Journal of Infectious Diseases

SAJID - 11: Manuscript Accepted for Publication, Sent to Editing

****
Ref. No.: 11
Manuscript title: IL-10 and TNF-α promoter region polymorphisms and susceptibility to urogenital schistosomiasis in young Zimbabwean children living in Schistosoma haematobium endemic regions
Journal: Southern African Journal of Infectious Diseases
****

Dear Dr Amos Marume

You will be pleased to know that your manuscript has been accepted for publication on 23-06-2020.

We would like to confirm that your manuscript has now been sent to our publishing department for finalisation.

Kindly note:
1. If you need to make contact with the publisher during the finalisation stage of your manuscript, kindly contact us per email or phone. Your new publisher contact will be Jana Venter, email: publishing@sajid.co.za and telephone extension: 507
2. The finalisation procedure works as follows:
(a) The first stage is the language editing that is returned to the corresponding author for review. This will be the final opportunity for the corresponding author to make text changes to the manuscript.
(b) At a later stage, the editorial staff will send the corresponding author one set of galley proofs, at which time the author will have two working days to mark any typographical errors.
3. Manuscript tracking is available on the submitting authors' journal profile. The submitting Author could visit their home page frequently to assess the stage of the manuscript.

Kind regards,
AOSIS: Tanien Botes
Phone +27 0219752602
6ts.srsupport@sajid.co.za

Appendix 9: Submission receipt - African Journal of Laboratory Medicine

aosis@ajlmonline.org Fri, Nov 22, 6:03 PM (23 hours ago)

to me

****
Ref. No.: 1138
Manuscript title: Cytokine TNF-α -308 G/A and IL-10 -819 C/T Promoter Region Single Nucleotide Polymorphisms Effects on Susceptibility to Urogenital Schistosomiasis in Pre-school Children in Zimbabwe
Journal: African Journal of Laboratory Medicine
****
Dear Dr Amos Marume

Your submission has been received by the journal and will now be processed in accordance with published timelines.

Processing time guidelines are available under the journal's 'About' section, however, please note that each submission is assessed on its individual merit and in certain circumstances processing times may differ.

You can check the status of your submission in three ways:
- Journal Website: login to your account at https://ajlmonline.org/index.php/ajlm/author/submission/1138.
- Publisher Enquiry Service: telephone numbers are +27(0)219752602 and/or 0861000381.
- Publisher FAQ and Email Service: visit the Publisher FAQ and Email service at https://publishingsupport.aosis.co.za/index.php

You will receive additional emails from the journal as your submission passes through the phases of the editorial process.

Kind regards,
AOSIS Publishing
African Journal of Laboratory Medicine

African Journal of Laboratory Medicine
https://ajlmonline.org

If you require immediate assistance, please contact the AOSIS Publishing:

Tel: RSA: 0861000381 - Intnl: +2721 975 2602
Fax: +27 (0)86 5004 974
Support email: publishing@aosis.co.za
Business hours are weekdays between 8:00am-16:30pm
Confidentiality: The information contained in and attached to this email is confidential and for use of the intended recipient. This email adheres to the email disclaimer described on www.aosis.co.za.

Appendix 10: Submission receipt - African Journal of Primary Health Care & Family Medicine

aosis@phcfm.org 5:12 PM (28 minutes ago)

to me

****
Ref. No.: 2313
Manuscript title: Overview of Schistosomiasis Chemotherapy using Praziquantel
Journal: African Journal of Primary Health Care & Family Medicine
****
Dear Mr Amos Marume

Your submission has been received by the journal and will now be processed in accordance with published timelines.

Processing time guidelines are available under the journal's 'About' section, however, please note that each submission is assessed on its individual merit and in certain circumstances processing times may differ.

You can check the status of your submission in three ways:
- Journal Website: login to your account at https://phcfm.org/index.php/phcfm/author/submission/2313.
- Publisher Enquiry Service: telephone numbers are +27(0)219752602 and/or 0861000381.
- Publisher FAQ and Email Service: visit the Publisher FAQ and Email service at https://publishingsupport.aosis.co.za/index.php

You will receive additional emails from the journal as your submission passes through the phases of the editorial process.

Kind regards,
AOSIS Publishing
African Journal of Primary Health Care & Family Medicine

African Journal of Primary Health Care & Family Medicine
This journal is available at https://phcfm.org

If you require immediate assistance, please contact the AOSIS Publishing:

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Fax: +27 (0)86 5004 974
Support email: publishing@aosis.co.za
Business hours are weekdays between 8:00am-16:30pm