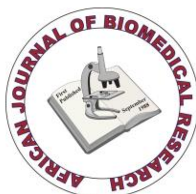

www.ajbrui.org

Afr. J. Biomed. Res. Vol. 23 (September, 2020); 375- 380

Research Article

SLC11A1 Gene Polymorphism in Adults Co-Infected with Helminth and Latent Tuberculosis in Yewa, Ogun State

Adepoju P.O.[1,2], Onile O.S.[1], Awobode T.[1], Cadmus S.I.B.[3], Anumudu C.I.A.[1]

[1]*Cell Biology and Genetics Unit, Department of Zoology, University of Ibadan, Ibadan, Nigeria.*
[2]*Department of Medical Laboratory Science, Babcock University, Ilishan-Remo, Ogun state, Nigeria.*
[3]*Department of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.*

ABSTRACT

Mutations in the 3'UTR and D543N regions of the solute carrier 11a1 protein (SLC11A1) gene have been found to strongly increase the risk of several diseases caused by intracellular organisms such as *M. tuberculosis.* The aim of this study was to screen for polymorphisms in the 3'UTR and D543N regions of SLC11A1 gene with the goal of understanding the genetic dynamics of tuberculosis and schistosomiasis co-infection in a Nigerian adult population. A cross-sectional study was carried out with 185 participants who were screened for intestinal and urinary helminthiases using microscopic examination of stool and urine respectively; latent tuberculosis using skin tuberculin test; and active tuberculosis using sputum microscopy. PCR-RFLP analyses were carried out on extracted DNA for detection of SLC11A1 gene polymorphisms. Participants filled questionnaires from which information on awareness, clinical and family histories and lifestyles were obtained. There were no polymorphisms observed. 32% had urinary schistosomiasis and 0.1% had intestinal helminthiasis suggesting that both types of infections could occur independently in the same population. The prevalence of coinfection with schistosomiasis and tuberculosis was 6.5%. This observation suggests an immunomodulation during schistosomiasis and latent tuberculosis co-infection. The absence of polymorphisms did not support the hypothesis that co-infection with schistosomiasis and latent tuberculosis might play a role as a risk factor during the development of active tuberculosis.

Keywords: *Latent tuberculosis, schistosomiasis, co-infection, polymorphisms, SLC11A1, NRAMP1*

**Author for correspondence: Email: adepojupaul@gmail.com; Tel: +2348060887107*

Received: January,2020; Accepted: December, July, 2020

Abstracted by:
Bioline International, African Journals online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius

INTRODUCTION

Both tuberculosis and human helminthiasis are diseases that exhibit an extensive distribution, causing serious harm to humans. In 2012, there were an estimated 436 million people at risk of *Schistosomiasis haematobium* infection in Sub-Saharan Africa, of which 112 million were infected. In addition, an estimated 393 million people were at risk of *Schistosomiasis mansoni* infection, out of which 54 million were infected (WHO, 2013). These figures suggest that there is an overlap of endemic regions between tuberculosis and parasitic disease.
Investigators have demonstrated in clinical settings that helminth infections may be associated with an increased incidence of *Mycobacterium tuberculosis* disease. Elias *et al* (2006) and Tristao-Sa (2002) showed an association between active pulmonary tuberculosis and helminths. However, such association studies do not confirm cause-effect relationship.

The co-infection of tuberculosis and helminthiasis has been reported in many studies for almost 70 years and great achievements have been gained in the separate fields of tuberculosis and helminth disease control and prevention (WHO, 2013). As at 2012, several epidemiological surveys of helminthiasis and schistosomiasis co-infection in hospitals or communities have been carried out (Choi *et al.,* 1984; Abate *et al.,* 2012), some of these studies showed that the host's immune response was modified in the co-infection situation (Resende *et al.,* 2007; Mendez-Samperio, 2012; Rafi *et al.,* 2012; Ferreira *et al.,* 2012).

To determine the susceptibility to active *Mycobacterium tuberculosis* by certain gene polymorphisms, the candidate gene would be the gene that plays active and essential roles in disease initiation, pathogenesis and/or pathology. Several genes that play roles in the disease pathophysiology have been studied but the one with the strongest potential according to

Soborg *et al* (2007) is the gene encoding solute carrier 11a1 protein gene (SLC11A1, formerly NRAMP1). It has polymorphisms that positively correlate with susceptibility to tuberculosis infection in Africans (Bellamy *et al.,* 1999), West Africans (Bellamy *et al.,* 1998) and Gujarati Asians in West London (Wilkinson *et al.,* 2000).

The broad objective of this study is to determine the influence of genetic polymorphisms on the pathophysiology of co-infections in Nigeria; and the specific objectives are to determine the prevalence of active and latent tuberculosis in the study population; the prevalence of urinary and intestinal helminthiases; the presence and frequency of SLC11A1 gene polymorphisms; and the association of these polymorphisms with the status of tuberculosis and helminthiasis infection.

MATERIALS AND METHODS

Study Design: For this cross-sectional study, 185 adults above 20 years were recruited. Members of the community were enlisted and screened for helminth worms and *M. tuberculosis* using informed consent procedures. Questionnaires were developed in English and Yoruba languages to assist in acquiring information on family history of tuberculosis, helminthiasis, and for socio-demographic data, age, sex and lifestyles.

Ethical issues:
Subjects included in the study gave informed consent and ethical approval for the study was obtained from the joint UI/UCH Ethical Review Committee (NHREC/05/01/2008a). Individuals included in the test study had helminth and latent tuberculosis co-infection; resident in the study area; provided blood, sputum, stool and urine samples needed for the study and were not infected by, or receiving treatment for HIV.

Test for intestinal helminths: Wide mouthed universal container was given to each of the participants for fecal sample collection. Specimens were examined grossly for the presence of blood, mucus, intact worms, and/or worm segments. Following centrifugation of sieved feaces in zinc sulphate floatation medium, the entire area under the coverslip was examined microscopically at X100 and X400 magnifications. The presence of eggs, cysts and/or adult forms of the worms was taken as a positive diagnosis.

Test for urinary helminths: 10ml of the urine sample was centrifuged at 5000 rpm for 5 minutes. The supernatant was discarded to leave sediment which was transferred to the centre of a clean grease-free glass slide to which was added a cover slip. This was mounted on a light microscope and examined at ×40 objective to identify *Schistosoma haematobium* ova which was characterised with a terminal spine. Those positive for urinary schistosomiasis infection were treated with the drug Praziquantel.

Test for Latent Tuberculosis Infection (LTBI): The targeted Tuberculin Skin Test (TST), also called the Mantoux test, is the most accepted method of LTBI screening (ATS and CDC, 2010). Briefly, 0.1 mL (5 tuberculin units) intradermal injection of purified protein derivative was placed on the volar surface of the forearm. This raised an initial weal of 6 to 10 mm in diameter. Reaction size was determined after 48 to 72 hours, although positive reactions often remained for up to one week.

Test for active tuberculosis infection: Slides were prepared from sputum samples in duplicates, a strategy already validated in the literature. Sputum samples were subsequently analyzed with the Ziehl-Neelsen technique on concentrated specimens as described by ATS and CDC (2000).

DNA extraction: Blood samples were collected on Whatman filter paper and allowed to dry before processing. Each 10mm filter paper punch was placed in an Eppendorf tube, and soaked in 65 microlitre of TE buffer; the tube and its content were incubated at 50°C for 15 minutes. At end of the incubation period, the punches were pressed gently at the bottom of the tube several times, using a new pipette tip for each punch and heated at 97°C for 15 minutes to elute the DNA. The liquid condensing on the lid and the wall of the tubes was removed by a short centrifugation (2−3 seconds). The DNA extract was kept at 4°C for use within a few hours or stored at −20°C.

Polymerase Chain Reaction (PCR): PCR was performed in a total volume of 25μl of solution, containing 0.1μg of genomic DNA, 5μl free Mg^{2+}10X PCR buffer (Roch Diagnostic GmbH, Germany), 200μM dntps, 1.5mM $MgCl_2$, 0.4μM of each primer and 2.5 units Taq DNA polymerase (Roch). Thermal cycling was performed on a TC-412 device (Techne, Cambridge, UK). For D543 and 3'UTR SLC11A1 polymorphism, the reaction was allowed to continue for 3 min at 94ºC, denaturation for 1min at 94ºC, annealing for 1min at 55ºC, extension for 1min at 72ºC repeated at 30 cycles and 3min at 72ºC, and then stored at 4ºC. With INT-4, annealing was done at 56ºC for 1min according to Kim *et al* (2003). All other procedures were identical.

The primers' characterization was as follows: 5'-CTC TGG CTG AAG GCT CTC C-3' and 5'-TGT GCT ATC AGT TGA GCC TC. For D543N and 3'UTR 5-GCA TCT CCC CAA TTC ATG G-3' and 5'-AAC TGT CCC ACT CTA TCC TG-3' 15.A region of 244bp for D543 and 3'UTR, and 623bp of DNA fragment for INT-4 was amplified.

Amplification parameters: An initial denaturation at 94°C for 5 min followed by 35 cycles each of denaturation at 94°C for 1 min, annealing at 68°C for 1.5 min, and extension at 72°C for 2 min. The extension step in the 35th cycle was for 10 min before the PCR products were stored at 4°C.

Restriction Fragment Length Polymorphism (RFLP): *ApaI* restriction enzyme was used for INT-4 region of the SLC11A1 gene; *AvaII* restriction enzyme was used for D543N and *FOKI* restriction enzyme was used for 3UTR. Digested products were ran on 8% polyacrylamide gel, and stained with silver nitrate as applied by Abe *et al* (2003).

Data analysis
The continuous variables were expressed as a group means ± standard deviation (SD). The primary variable was polymorphism in SLC11A1 gene in co-infected individuals and control subjects. Secondary variables included gender, age, contact and duration of infection. Statistical analyses were performed when appropriate. All P-values was two-tailed. A P-value <0.05 was considered statistically significant.

Variants at 3 polymorphisms (3' UTR, INT4, D543N) in the *NRAMP1* gene were compared between tests and controls with Fisher's Exact test. To assess the differences in demographic characteristics, Chi-square (for categorical data) and t test (for continuous data) were used. Deviations from Hardy-Weinberg Equilibrium (HWE) were assessed using the chi-square test. Subsequently, the 3 *NRAMP1* polymorphisms were independently associated with the co-infection through logistic regression analysis after adjustment for age and sex.

RESULTS

The prevalence of schistosomiasis, tuberculosis and co-infection are shown in Figure 1 below.

Intestinal helminths*:* none were positive for active infection in both normol saline and iodine wet preparations. There were 5 blood-stained stool samples but the absence of eggs or ova of *S. haematobium,* and the well-formed nature of the stool suggested that the presence of blood may be as a result of a cut in the anal region of the affected patients.

Urinalyses: 38 samples tested positive for *S. heamatobium* representing 20.9% of the test population. Out of the 38 participants that were positive for *S. haematobium,* diagnostically substantial leukocyturia were identified in 21 (55.3%) participants. Only 2 participants that were negative had similar amounts of pus cells in their urine sediments.
The proportion of participants using water from various sources for daily chores is also shown in Figure 2.

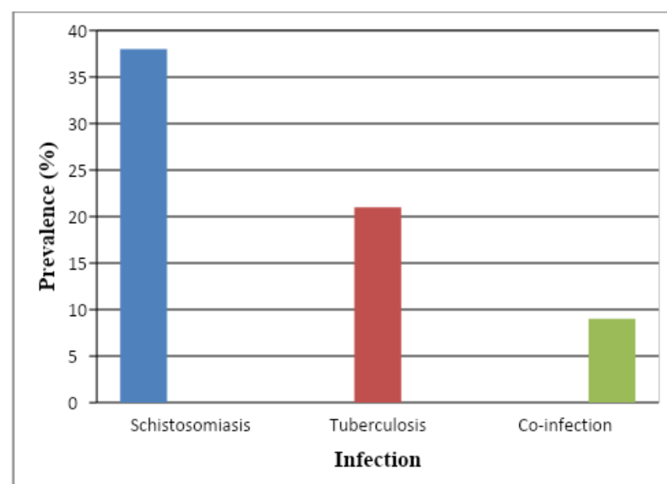

Figure 1:
Prevalence of schistosomiasis, tuberculosis and co-infection in the study population

The prevalence of the various forms of interventions in the treatment of tuberculosis and schistosomiasis is also shown in Figure 3 below.

Calcium oxalate crystals as shown in Table 1 below were detected and identified in 12 participants out of which 7 (58.3%) were positive for schistosomiasis but was not statistically significant for *S. haematobium*. Haematuria was detected in 8 female participants of which only 1 (12.5%) was positive for schistosomiasis.

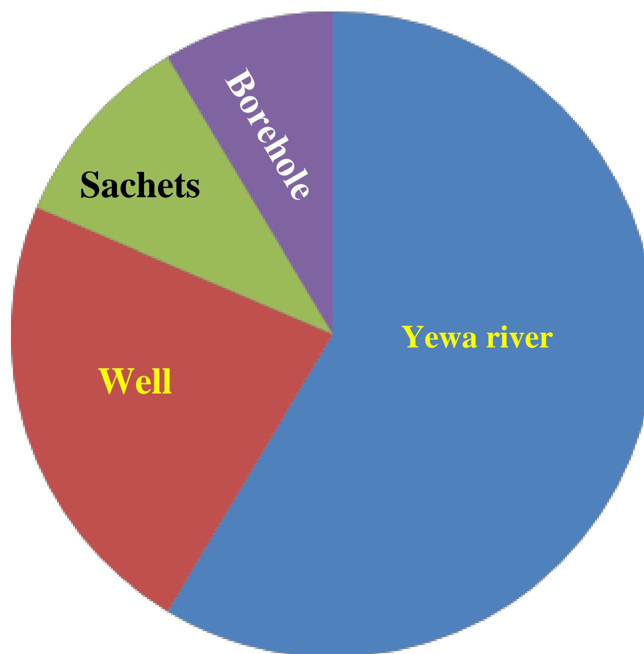

Figure 2:
Proportion of participants using water from various sources for daily chores

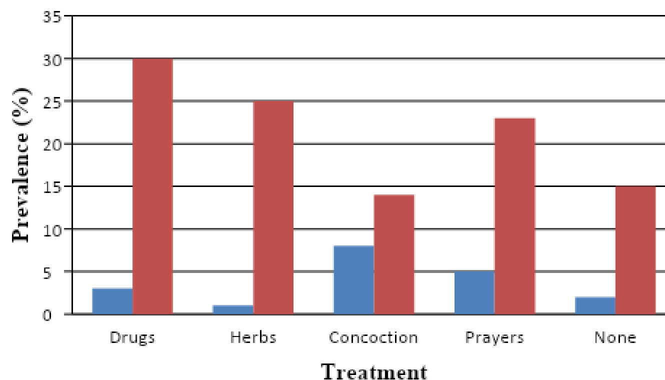

Figure 3:
Preferred forms of interventions in the treatment of tuberculosis and schistosomiasis

Table 1:
Incidences of leukocyturia, haematuria and urinary calcium oxalate crystals in *S. haematobium* positive and negative participants.

Components	Schistosomiasis positive	Schistosomiasis negative	Total
Leukocyturia	21	2	23
Calcium oxalate crystals	7	5	12
Haematuria	1	7	8

Urine biochemistry: The average pH value in both groups (*S. haematobium* positive and negative) was 5.5, both groups had similar average glucose values with only 2 individuals in each group presenting with glycosuria. However, both groups showed dissimilar protein values. While 12 individuals that

were positive for *S. haematobium* (31.57%) had proteinuria, only 5 individuals that were negative (3.52%) presented with proteinuria. The proteinuria levels correlated moderately (r = 0.6) with schistosomiasis.

Test for active tuberculosis*:* Only 4 had scanty bacteria that were fewer than World Health Organization's standard for positive active tuberculosis diagnosis using slide microscopy.
*SLC11A1 Gen*e *polymorphisim:* The normal individual D543N gave an undigested 244bp while a mutant individual gave 2 fragments. The normal individual 3'UTR gave an undigested 244bp while a mutant individual gives fragments. There were no mutant polymorphisms for D543N and 3'UTR as shown in Plates 1 and 2 respectively.

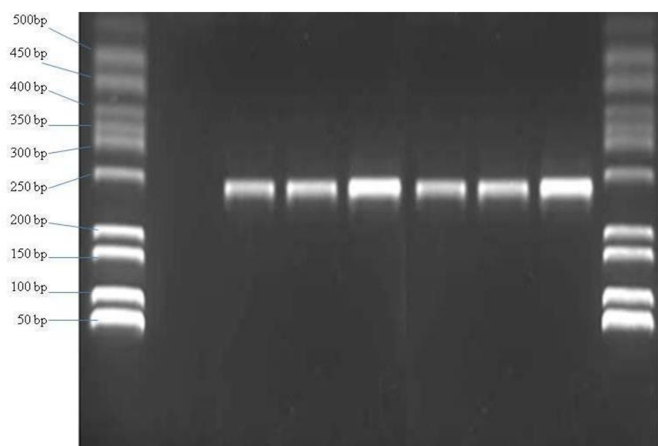

Plate 1:
PCR-RFLP profiles for the D543N region of the SLC11A1 gene. Lanes M1 and M2 are the 50kb plus DNA ladders. Lanes 2 to 7 represent the undigested PCR-products. There were no amplifications in Lane 1 and the procedure had to be repeated following which similar single bands of undigested PCR products were recorded.

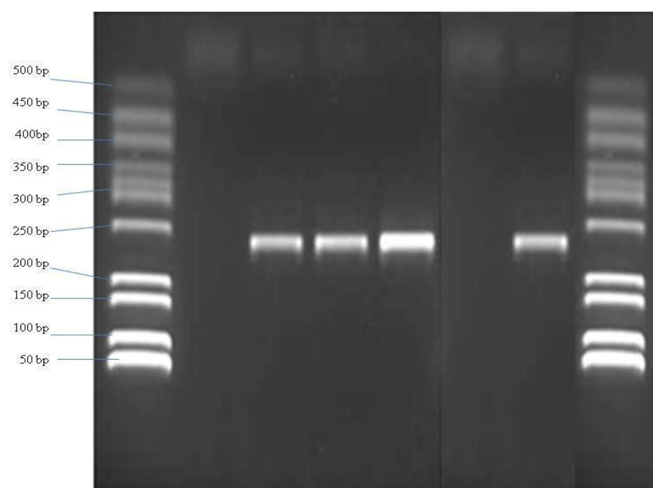

Plate 2:
PCR-RFLP profiles for the 3'UTR region of the SLC11A1 gene. Lanes M1 and M2 are the 50kb plus DNA ladders. Lanes 2, 3 and 4 represent the undigested PCR-products from DNA extracted from participants that were positive for schistosomiasis, tuberculosis and co-infection respectively. There were no amplifications in Lanes 1 and 5 and the procedure had to be repeated following which similar single bands of undigested PCR products were recorded.

Table 2:
(a) Intensity of *S. haematobium* ova in latent TB-positive and negative participants

Quantity description	TB-positive	TB-Negative	Total
Light (≤ 5 ova/10ml of urine)	20	2	**22**
Moderate (≤ 20 ova/10ml of urine)	8	4	**12**
Heavy (≥ 50 ova/10ml of urine)	7	3	**10**
Total	**35**	**9**	**44**

(b) Prevalence of latent tuberculosis infection (LTBI) positivity determined by formation of weal (>5mm) following exposure to *M. tuberculosis* antigen using Mantoux test in participants infected and uninfected with *S. haematobium*

S. haematobium	LTBI Negative	LTBI Positive
Positive	19%	6.5%
Negative	67.7%	48.4%

DISCUSSION

Nigeria has high prevalence figures for both schistosomiasis (Bala *et al.,* 2012) and latent tuberculosis (Adeiza, 2011). Studying both diseases individually could be difficult due to risks of confounding factors such as infection with HIV (Ignatowicz *et al.,* 2012).

Even though the popular Yewa River has been linked with schistosomiasis in the study community (Salawu and Odaibo, 2013), as high as 82% of study participants said they heavily depend on the river as a source of water for domestic purposes such as drinking, washing, bathing, swimming and several others. The high dependence on the river is as a result of the non-availability of a more readily available safer source of water for daily chores. This study has shown that the major source of water for daily chores is a major factor in schistosomiasis infection as the incidence of schistosomiasis increased with increased number of participants that used water from the river.

Responses to questions asked in the questionnaire showed that in the study population, the levels of awareness on health issues specifically schistosomiasis, tuberculosis and HIV/AIDS was low with less than 20% of respondents having basic information on endemic diseases. Furthermore, the levels of awareness were not strongly associated with the type of religion, participants' age group, levels of education, marital status or standard of living.

Data collected via questionnaires (Figure 3) showed that more people used pharmaceutical drugs for the treatment of schistosomiasis than other forms of treatment while more people said they used local concoctions than pills in the treatment of symptoms of tuberculosis. One of the reasons why more participants preferred pharmaceutical drugs was the availability of drugs for the treatment of schistosomiasis as previously researchers in the study area had made Praziquantel® available for the treatment of individuals that tested positive for *S. haematobium.* This would have created awareness in the study area on the availability and efficacy of pharmaceutical agents in the treatment of schistosomiasis. For

tuberculosis on the other hand, more respondents said they used concoctions for TB therapy. This could be due to the lack of easy access to tuberculosis diagnosis and treatment facilities as residents have to visit a distant community for TB healthcare services.

No patient tested positive for intestinal helminthiasis using macroscopic and microscopic fecal examination methods. This is supported by the absence of classical symptoms of intestinal schistosomiasis infection such as abdominal pain, diarrhoea, and the presence of blood in the stool.

Intestinal helminth worms are mostly acquired through eating unwashed vegetables and uncooked meat such as pork, venison and beef; however, in the study area, while vegetables may not be adequately washed, they are often well cooked before they are eaten. Furthermore, residents in the community are highly religious thus making the consumption of pork rare. Like in other areas in southwest Nigeria, consumption of uncooked meat is very rare in the study area. Before meat is consumed, it is often taken through several processes of boiling and frying which occur at very high degrees of temperatures. The high incidence of urinary schistosomiasis and low incidence of intestinal helminthiasis suggest that both types of infections could occur independently as long as reservoirs for both types of infections are readily available.

Leukocyturia found in individuals who tested positive for schistosomiasis is in line with the findings of Oliveira *et al.* (2011) and suggests active roles for leukocytes during the infection. Roles such as priming of endothelial cells have been suggested since schistosomiasis increases vascular permeability and endothelial cell-leukocyte interaction *in vivo* and *in vitro.* The finding also suggests that leukocyturia could be an im*portant* diagnostic and prognostic tool; more than haematuria during schistosomiasis since it could be the bridge that links the infection to the immunological processes discussed by Elias *et al.* (2011). It has also been reported by other researchers who carried out similar studies in Nigeria (Adeyeba and Ojeaga, 2002) although they reported significantly high incidence of haematuria among children, but not in adults.

The presence of calcium oxalate crystals in urinary deposits suggest that some participants who were positive for schistosomiasis may be receiving some form of treatment for the disease or some types of other ailments such as non-communicable diseases or non-infectious conditions they did not disclose, despite the fact that they had not been tested before; similar crystals were found in urinary samples collected from some participants who tested negative for both infections and are apparently healthy.

According to the results of the urinary pH analyses, both groups of individuals with schistosomiasis and individuals who were negative for the urinary helminth had similar average pH values. This suggests that the S. haematobium parasite may not be capable of cell lysis unlike *S.mansoni where* maximum schistosome-induced hemolysis occurs when the worm comes in contact with red blood cells in a low pH (pH 5.1), high temperature (37ºC) environment for a short time (30 minutes), after which hemolysis occurs at both pH 7.5 and 5.1 (Kasschau *et al.,* 1995).

Although the moderate proteinuria in individuals with schistosomiasis is less specific and of little diagnostic importance by itself, when combined with leukocyturia and haematuria, the sensitivity could exceed 90% as also reported by Doehring *et al.,* 1985.

The observation of higher frequency of positivity of tuberculin reaction in individuals who are coinfected with schisosomiasis and tuberculosis compared with the lower frequency of positivity of tuberculin reaction in individuals that tested negative for schistosomiasis (Table 2) suggests a strong correlation, immunomodulation, at the advanced stages of schistosomiasis and latent tuberculosis. Elias *et al.* (2011) and other researchers suggested that the strong correlation between schistosomiasis and tuberculosis is indicative of an interaction – suggestively genetic – between the pathways of both TH1 and TH2 responses that are predominant in the immunologic responses to schistosomiasis and latent tuberculosis respectively.

According to Schurr (2011), evidence of genetic involvement in tuberculosis pathogenesis has come primarily from twin studies and risks to first-degree relatives of cases. In addition, inferences of strong genetic influences have come from anecdotal accounts of socially prominent families, population variation in tuberculosis incidence and susceptibility to infection, and secular changes in tuberculosis severity, incidence and mortality inferred from historical information of contact between different populations, as well as accidental inoculation of vaccines with *M. tuberculosis*. SLC11A1 gene however remains the candidate gene for active tuberculosis susceptibility studies and this study provides further affirmation for its continual usage in various population studies on tuberculosis. The two regions of the SLC11A1 gene that were selected for study in this work were amplified in the target population with strong bands visualized on gel electrophoresis. But the absence of polymorphisms following RFLP analyses and visualization on PAGE suggests that co-infection with *S. haematobium* and latent tuberculosis may not result in diagnostically important mutations in the two regions on the SLC11A1 gene which could increase an individual's risk of developing active tuberculosis.

Since this study is focused on adult members of the population, increased age may have played an important role in the participants' mutation repair fidelity and capacity as demonstrated in several other genetic studies including Reichel *et al.,* (2001) which found different sets of mutations in infants, children and adults. More regions of the gene should be sequenced, analyzed and studied. This is due to the reported population-wide variations in the activity and sensitivity of the various regions of the SLC11A1 gene. This pilot study only tested 185 samples which may be too small for conclusive population genetics study of polymorphisms. It is therefore recommended that similar studies should be carried out using more diverse and larger study populations.

Genome-wide association studies (GWAS) are also recommended since this study confirms the involvement of at least two immunologic pathways. Similar or dissimilar immunogenetic reactions could be responsible for immunologic variations and may be indicative of co-infection. The results of this study suggest that a combination of proteinuria, leukocyturia and haematuria is a better diagnostic and prognostic index in schistosomiasis diagnosis and research.

The results obtained from the polymerase chain reactions carried out on the various regions of the SLC11A1 gene suggests that the procedure may serve diagnostic purposes in active and latent tuberculosis infections.

In conclusion, the relationship between latent tuberculosis infection and helminthiasis is important in understanding the pathophysiology of the co-infection and the roles played in the development of active tuberculosis. The high prevalence of urinary helminths and co-infection with *M. tuberculosis* reported in the study area represent an infection burden and indicate an immunomodulation in the co-infection.

The absence of polymorphisms did not support the hypothesis that co-infection with schistosomiasis and latent tuberculosis might play a role as a risk factor during the development of active tuberculosis. Thus, it will be important to carry out more studies to clarify the biological underpinnings of the relationships reported in this study as well as further immunological and population genetics studies to focus on the significance of these interactions.

REFERENCES

Abate, E., Belayneh, M., Gelaw, A., Idh, J., Getachew, A., Alemu, S., Diro, E., Fikre, N., Britton, S., Elias, D., Aseffa, A., Stendahl, O., Schon, T. (2012): The Impact of Asymptomatic Helminth Co-Infection in Patients with Newly Diagnosed Tuberculosis in North-West Ethiopia. PLoS ONE 7(8): e42901

Abe, T., Iinuma, Y., Ando, M., Yamamoto, T., Nakashima, K. (2003): NRAMP1 polymorphisms, susceptibility and clinical features of tuberculosis. *J Infect*; 46 (4): 215- 20.

Adeyeba, O.A., Ojeaga, S.G.T. (2003): Urinary schistosomiasis and concomitant urinary tract pathogens among school children in metropolitan Ibadan, Nigeria. *African Journal of Biomedical Research.* 5 (3): 112-116

ATS, CDC. (2000): Targeted tuberculin testing and treatment of latent tuberculosis infection. *Am J Respir Crit Care Med*. 161(4 pt 2):S221–S247.

Bala, A.Y., Ladan, M.U., Mainasara, M. (2012): Prevalence of urinary schistosomiasis in Abarma Village, Gusau, Nigeria: A preliminary investigation. *Science World Journal.* 7(2):121-126

Bellamy, R., Ruwende, C., Corrah, T., McAdam, K.P., Thursz, M., Whittle, H.C., Hill, A.V. (1999): Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the Vitamin D receptor gene. *J. Infect. Dis*. 179, 721–724.

Bellamy, R., Ruwende, C., Corrah, T., McAdam, K.P., Whittle, H.C., Hill, A.V. (1998): Variations in the NRAMP1 gene and susceptibility to tuberculosis in West Africans. *N. Engl. J. Med.* 338, 640–644.

Choi, W.Y., Yoo, J.E., Kim, W.G., Yun, B.H., Kim, S.G., Yoo, W.H. (1984): Prevalence of intestinal helminthic infections and skin tests for paragonimus and clonorchis in tuberculosis patients. *Kisaengchunghak Chapchi* **22:**209-214.

Doehring, E., Ehrich, J.H., Vester, U., Feldmeier, H., Poggensee, U., Brodehl, J. (1985): Proteinuria, hematuria, and leukocyturia in children with mixed urinary and intestinal schistosomiasis. *Kidney Int.* 28(3):520-525

Elias, D., Mengistu, G., Akuffo, H., Britton, S. (2006): Are intestinal helminths risk factors for developing active tuberculosis? *Trop Med Int Health*; 11:551–8.

Elias, D., Wolday, D., Akuffo, H., Petros, B., Bronner, U., Britton, S. (2011): Effect of deworming on human T cell responses to mycobacterial antigens in helminth-exposed individuals before and after bacille Calmette-Guerin (BCG) vaccination. *Clin. Exp. Immunol.* 123:219–225

Ferreira, A.P., Aguiar, A.S., Fava, M.W., Correa, J.O., Teixeira, F.M., Teixeira, H.C. (2002): Can the efficacy of bacille calmette-guerin tuberculosis vaccine be affected by intestinal parasitic infections? *J Infect D*, **186:**441-442.

Ignatowicz, L., Mazurek, J., Leepiyasakulchai, C., Sköld, M., Hinkula, J., Källenius, G., Pawlowski, A. (2012): *Mycobacterium tuberculosis* Infection Interferes with HIV Vaccination in Mice. *PLOS ONE.* 7(7): e41205.

Kasschau, M.R., Byam-Smith, M.P., Gentry, D.S., Watson, F.N. (1995): Influence of pH and temperature on hemolysis by adult Schistosoma mansoni membranes. *J Exp Zool.* 271(4):315-22

Kim, J.H., Lee, S.Y., Lee, S.H., Sin, C., Shim, J.J., In, K.H. (2003): NRAMP1 genetic polymorphisms as a risk factor of tuberculous pleurisy. *Int J Tuberc Lung Dis*; 7 (4): 370- 5.

Mendez-Samperio, P. (2012): Immunological mechanisms by which concomitant helminth infections predispose to the development of human tuberculosis. *Korean J Parasitol*, **50:**281-286.

Rafi, W., Ribeiro-Rodrigues, R., Ellner, J.J., Salgame, P. (2012): Coinfection-helminthes and tuberculosis. *Curr Opin HIV AIDS.* 7(3):239-44.

Reichel, M., Gillert, E., Angermüller, S., Hensel, J.P., Heidel, F., Lode, M., Leis, T., Biondi, A., Haas, O.A., Strehl, S., Panzer-Grümayer, E.R., Griesinger, F., Beck, J.D., Greil, J., Fey, G.H., Uckun, F.M., Marschalek, R. (2001): Biased distribution of chromosomal breakpoints involving the MLL gene in infants versus children and adults with t(4;11) ALL. *Oncogene*20(23):2900-2907

Resende S., Co, T., Hirsch, C.S., Toossi, Z., Dietze, R., Ribeiro-Rodrigues, R. (2007): Intestinal helminth co-infection has a negative impact on both anti-Mycobacterium tuberculosis immunity and clinical response to tuberculosis therapy. *Clin Exp Immunol*, **147:**45-52.

Salawu, O., Odaibo O. (2013): Urogenital schistosomiasis and urological assessment of hematuria in preschool-aged children in rural communities of Nigeria. *Journal of Pediatric Urology.* xx, 1 – 6.

Schurr, E. 2011. The contribution of host genetics to tuberculosis pathogenesis. *Kekkaku* 86(1): 17-28

Soborg, C., Andersen, A.B., Range, N., Malenganisho, W., Friis, H., Magnussen, P., Temu, M.M., Changalucha, J., Madsen, H.O., Garred, P. (2007): Influence of candidate susceptibility genes on tuberculosis in a high endemic region. *Molecular Immunology* 44:2213–2220

Tristao-Sa, R., Ribeiro-Rodrigues, R., Johnson, L.T., Pereira, F.E., Dietze, R. (2002): Intestinal nematodes and pulmonary tuberculosis. *Rev Soc Bras Med Trop* 35:533-535

WHO. 2013: Fact sheet on schistosomiasis. World Health Organization, Geneva

Wilkinson, R.J., Llewelyn, M., Toossi, Z., Patel, P., Pasvol, G., Lalvani, A., Wright, D., Latif, M., Davidson, R.N. (2000): Influence of Vitamin D deficiency and Vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. *Lancet* 355, 618– 621.