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The Impact of Schistosomiasis Co-infections on Immune Responses in Leprosy

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The Impact of Schistosomiasis Co-infections on Immune Responses in Leprosy

By

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Bachelor of Science
University of Michigan
2017

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Abstract

The Impact of Schistosomiasis Co-infections on Immune Responses in Leprosy

By Laura E. Kochlefl

Leprosy is an ancient neglected tropical disease caused by *Mycobacterium leprae*. India and Brazil carry the highest burden of disease, much of which is concentrated in hyperendemic areas with more than 40 new cases per 100,000 people annually. Helminth co-infections, like schistosomiasis, can cause immune dysregulation and may contribute to disease transmission. This paper analyses data from a case-control study performed in municipalities Governador Valadares and Mantena in the state of Minas Gerais, Brazil. A total of 79 leprosy patients and 178 healthy adults were recruited and administered questionnaires. Schistosomiasis was diagnosed from stool samples using Keto Katz methods. Levels of Th1 cytokines IFN-γ and TNF-α, Th2 cytokines IL-4 and IL-10, and Th17 cytokine IL-17 were assessed from blood samples using a cytometric bead array and stimulated with *M. leprae*. Descriptive, one-way ANOVA and ANCOVA analyses were performed. Significant differences were detected in levels of IFN-γ, and TNF-α between the four groups when controlling for age, sex, and vitamin D deficiency. Less IFN-γ was detected in leprosy patients with schistosomiasis co-infections, while TNF-α was found to be increased in this group. Levels of IL-10 were found to be higher in leprosy patients with schistosomiasis co-infections while no difference was found in IL-4 levels between groups. Average levels of IL-17 were found to be lower in individuals with schistosomiasis co-infections. These results support the theory that schistosomiasis co-infections upregulate the Th2 immune response, and downregulate the protective Th1 immune response to *M. leprae,* potentially making individuals more susceptible to disease. These findings suggest individuals living in areas endemic for schistosomiasis and leprosy may be at a higher risk for leprosy and open up the potential for new avenues to be explored in transmission control efforts.

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I. Introduction

Leprosy is an ancient neglected tropical disease that continues to cause significant disability throughout the world (Rodrigues & Lockwood, 2011). After the development of a multi-drug therapy, global leprosy prevalence has decreased significantly, however incidence rates persist in many countries. In 2018, 208,619 new cases were reported globally (WHO, 2019a). Brazil reported the second highest number of incident cases in 2018, with 28,660 new cases (WHO, 2019a). This amounts to 93% of the total leprosy incident cases in the Americas (WHO, 2019a). Many of the incident cases occur in hyperendemic areas, as defined by more than 40 cases per 100,000 people. Transmission in these areas is still poorly understood.

Leprosy is a bacterial disease caused by *Mycobacterium leprae* (*M. leprae*). The disease is thought to spread primarily through person to person transmission via respiratory droplets (Rodrigues & Lockwood, 2011). However, it has been estimated that only 5-10% of individuals exposed to leprosy develop the disease (Lockwood & Saunderson, 2012). This appears to be at odds with the development of hyperendemic areas. Additionally, it has been reported that a majority of new cases in hyperendemic areas in Espirito Santo, Brazil were not contacts of a known leprosy case (Deps et al., 2006). A greater understanding of sociocontextual and environmental drivers of transmission is needed to address leprosy transmission in these areas.

A number of sociocontextual and environmental factors have been found to have spatial and statistical associations with leprosy transmission including crowding, low socioeconomic status, malnutrition, and poor access to sanitation facilities(Cabral-Miranda, Chiaravalloti Neto, & Barrozo, 2014; Cury et al., 2012; Kerr-Pontes, Montenegro, Barreto, Werneck, & Feldmeier, 2004; Nobre et al., 2015). Helminths represent a particularly interesting potential risk factor for leprosy. Helminths have been found to be both spatially and temporally correlated with leprosy

incidence (Phillips et al., 2017). Helminth co-infections have also been associated with more infectious forms of the disease, which may result in increased transmission (L. Diniz, Zandonade, Dietze, Pereira, & Ribeiro-Rodrigues, 2002). This association has also been shown to be biologically plausible. There is evidence that helminth infections can cause dysregulation in the host immune system that increases the susceptibility of the host to mycobacterium infection (Chatterjee & Nutman, 2015). Helminth infection can induce the upregulation of the T-helper cell type 2 (Th2) immune response, which results in a downregulation of the T-helper cell type 1 (Th1) immune response responsible for protection against *M. leprae* infection (Chatterjee & Nutman, 2015). Cytokine expression associated with the Th2 immune response has been shown to be higher, and those associated with Th1 lower, in leprosy patients with helminth co-infections compared to patients with no co-infection (L. M. Diniz, Magalhaes, Pereira, Dietze, & Ribeiro-Rodrigues, 2010). In this study, these associations are further investigated looking at schistosomiasis co-infections in leprosy patients to better understand the formation of hyperendemic areas in Minas Gerais, Brazil, and inform future infection control strategies.

II. Literature Review

Background

Leprosy is an ancient disease caused by mycobacterium *M. leprae*. One of the earliest identified samples in humans was from Roman-era Egypt in 445 B.C.E. (Monot et al., 2009). However, leprosy was likely in circulation before this. Gene sequencing suggests that leprosy was spread from East Africa to Europe and then throughout the rest of the world via trade routes (Monot et al., 2009). Throughout its history leprosy has been the cause of an immense amount of stigma. Although typically not fatal, leprosy can cause permanent disability and disfigurement. Even after the introduction of a multi-drug therapy in 1981, leprosy remains a leading infectious cause of disability throughout the world, and a source of stigma in many countries (Rodrigues & Lockwood, 2011).

The global prevalence of leprosy has decreased since the therapy became available, however, incidence rates are not declining as expected (Rodrigues & Lockwood, 2011). An estimated 208,619 incident cases were reported to the World Health Organization in 2018 (WHO, 2019a). However, this is likely an underestimation due to underreporting and limited surveillance. The highest concentration of incident cases was reported in India followed by Brazil (WHO, 2019b). In 2018, Brazil accounted for 93% of leprosy incidence in the Americas with 28,660 new cases (WHO, 2019b).

Leprosy Disease Characteristics

M. leprae is an acid-fast mycobacterium that targets the peripheral nerves (White & Franco-Paredes, 2015). The disease is characterized by insensitive legions on the skin. Although the disease progresses slowly, without treatment it can cause permanent nerve damage in the affected areas (Rodrigues & Lockwood, 2011). Leprosy is typically categorized using the Ridley

Jopling criteria, which proposes five different forms of the disease (Ridley & Jopling, 1966). Tuberculoid leprosy, followed by borderline tuberculoid are generally considered less severe with limited *M. leprae* bacilli infection (Britton & Lockwood, 2004; Nath, Saini, & Valluri, 2015). Tuberculoid forms of leprosy fall under the clinical classification of paucibacillary (PB). A PB case would involve fewer skin lesions, may not have a positive result from a skin smear test, and is considered less infectious (WHO, 2019d). PB cases also have a stronger T cell-mediated immune response than other forms of the disease (Britton & Lockwood, 2004; Nath et al., 2015). Another distinct form of the disease is lepromatous leprosy, which may be clinically defined as the multibacillary (MB) form of leprosy (Britton & Lockwood, 2004; Nath et al., 2015). MB cases present with more skin lesions and tend to have positive results from a skin smear (WHO, 2019d). This is also characterized by a negligible cell-mediated immune response and is the more infectious form of the disease (Britton & Lockwood, 2004; Nath et al., 2015; WHO, 2019d). Clinical presentations between tuberculoid and lepromatous leprosy are categorized as borderline tuberculoid, mid-borderline, and borderline lepromatous leprosy (Nath et al., 2015).

Approximately 30-50% of leprosy patients may also experience leprosy reactions (Fairley et al., 2019; Rodrigues & Lockwood, 2011; White & Franco-Paredes, 2015). Reactions are inflammatory responses that are characterized by a worsening of symptoms during treatment (White & Franco-Paredes, 2015). Type 1 reactions, or reversal reactions, are more common in patients with borderline forms of leprosy and can cause inflammation, pain in the lesions, and neuritis (Nath et al., 2015; White & Franco-Paredes, 2015). Type 2 reactions, also known as erythema nodosum leprosum reactions, are more commonly seen in patients with lepromatous

forms of leprosy and can cause fever, malaise, inflammation, and the development of painful subcutaneous nodules (Nath et al., 2015; White & Franco-Paredes, 2015).

Leprosy Transmission

Leprosy transmission is still poorly understood. Person to person transmission via respiratory droplets is believed to be the primary mode of transmission (Rodrigues & Lockwood, 2011). Household contacts are reported to be at high risk (van Beers, Hatta, & Klatser, 1999). Increased leprosy incidence has also been associated with a high number of household contacts and crowding (Cabral-Miranda et al., 2014; Castro, Santos, Abreu, Oliveira, & Fernandes, 2016; Deps et al., 2006). However, person to person transmission alone may not explain the continued incidence rates. It's estimated that only 5-10% of people exposed to leprosy develop the disease (Lockwood & Saunderson, 2012). Additionally, many new cases occur in hyperendemic areas, categorized as having greater than 40 cases per 100,000 people. A study in Espirito Santo found that the majority of new cases in hyperendemic areas were not contacts of a leprosy case (Deps et al., 2006). What factors make some individuals more susceptible than others to leprosy, and what factors may be associated with geographic clusters is still being explored. However, the existence of hyperendemic areas despite the low transmission rate suggests that there may be a sociocontextual or environmental factor influencing leprosy transmission.

Socioeconomic Risk Factors

There is consistent and abundant evidence that leprosy is associated with poverty. High levels of inequality and poverty have been associated with increased leprosy risk and incidence (Chaves, Costa, Flores, & Neves, 2017; Kerr-Pontes et al., 2004; Murto et al., 2013; Nery et al., 2014). Multiple studies have found poverty to be spatially associated with higher leprosy incidence rates in Brazil (Chaves et al., 2017; Cury et al., 2012; Queiroz et al., 2010). A cross-

sectional ecological study in Pará State, Brazil, found a spatial correlation between social deprivation index and leprosy detection rate (Chaves, 2017). A spatial analysis in southeastern Brazil found an association between areas of high leprosy incidence and neighborhoods with low socioeconomic levels (Cury et al., 2012). Low Urban Quality Index has also been associated with higher leprosy incidence in spatial analyses in Espirito Santo (P. Sampaio, Bertolde, Maciel, & Zandonade, 2013). Decreased leprosy incidence has been associated with a middle to high income and land ownership (Wagenaar et al., 2015). Higher inequality in the distribution of income has been correlated with an increased relative risk of leprosy in Bahia, Brazil (Cabral-Miranda et al., 2014; Kerr-Pontes et al., 2004; Nery et al., 2014).

Several studies have found leprosy to be associated with illiteracy, lower education, and unemployment (Duarte-Cunha, Marcelo da Cunha, & Souza-Santos, 2015; Nery et al., 2014; Nobre et al., 2015). Increased risk of leprosy was also associated with illiteracy and lower education (Kerr-Pontes et al., 2006; Kerr-Pontes et al., 2004). Unemployment has also been considered and found to be associated with increased leprosy risk (Duarte-Cunha et al., 2015; Nery et al., 2014).

Other demographic characteristics such as age, sex, and race have also been assessed. A spatial analysis of leprosy mortality distribution in Brazil found older, racially black, and male populations were associated with higher leprosy mortality rates (Martins-Melo et al., 2015). Several other studies have shown a positive relationship between leprosy and age (de Andrade, Sabroza, & de Araújo, 1994; Feenstra, Nahar, Pahan, Oskam, & Richardus, 2011). One other study found no difference in leprosy risk by race (Kerr-Pontes et al., 2006).

Diet-Related Risk Factors

Multiple studies have reported food shortages as being associated with increased leprosy (Feenstra et al., 2011; Kerr-Pontes et al., 2006; Wagenaar et al., 2015). Food shortages experienced in the past year or at any time in the life of the participant have been associated with higher leprosy risk in Bangladesh (Feenstra et al., 2011; Wagenaar et al., 2015). This association has also been reported in Brazil (Kerr-Pontes et al., 2006). Higher food stocks were additionally found to be associated with a lower risk of leprosy (Wagenaar et al., 2015).

Diet quality also plays a role in leprosy risk. Higher diet diversity has been associated with lower leprosy risk (Wagenaar et al., 2015). Malnutrition was reported to be spatially correlated with leprosy in children in Brazil (Nobre et al., 2015). A study in Bangladesh found that there was a significant association between malnutrition in children aged 1-4 and leprosy (Sommerfelt, Irgens, & Christian, 1985).

Specific micronutrients may play a significant role in leprosy risk, and the innate immune response. Low concentrations of vitamin A have been reported lepromatous leprosy patients(Lima, Roland Ide, Maroja Mde, & Marcon, 2007) Additionally, a study on food consumption in leprosy patients and household contacts shows a majority of leprosy patients and their household contacts consuming insufficient vitamin A (Passos Vázquez et al., 2014).

Several studies have examined vitamin D deficiency and its role in leprosy (Lu'o'ng & Hoàng Nguyễn, 2012; Passos Vázquez et al., 2014). There are numerous genetic and biological factors for which the vitamin D pathway and leprosy are associated, and evidence to support Vitamin D having a significant effect on the development and expression of leprosy (Lu'o'ng & Hoàng Nguyễn, 2012; Passos Vázquez et al., 2014). Certain polymorphisms of the Vitamin D receptor gene may be associated with the multibacillary form of leprosy (Pepineli et al., 2019). In

India, individuals with Type 2 leprosy reactions have been shown to have decreased expression of Vitamin D receptors and severely lowered vitamin D3 levels compared to healthy controls (Mandal et al., 2015). In a recent case-control study in Minas Gerais Brazil, Fairley et. al. reported more than half of the leprosy patients in their study were deficient in vitamin D (Fairley et al., 2019).

Environmental Risk Factors

Poor living conditions have been associated with leprosy (Nery et al., 2014; Queiroz et al., 2010). In a case-control study in Ceará, Brazil, an increased risk of leprosy was associated with living in a house with a sand or mud floor(Kerr-Pontes et al., 2006). Having access to waste disposal services and a sewage system has been associated with decreased prevalence of leprosy in Brazil (Castro et al., 2016; Duarte-Cunha et al., 2015; Freitas & P. Garcia, 2014; Murto et al., 2013; Nobre et al., 2015). Household access to clean water has additionally been associated with lower leprosy incidence (Castro et al., 2016; de Andrade et al., 1994; Duarte-Cunha et al., 2015; Kerr-Pontes et al., 2004).

Urban conditions have also been studied as a risk factor for leprosy with mixed results. Several studies have found an association with leprosy and increased urbanization(Cabral-Miranda et al., 2014; Kerr-Pontes et al., 2004). Migration into urban areas from rural areas has also been found to be associated with higher rates of leprosy (Cabral-Miranda et al., 2014; Murto et al., 2013). Another study found that leprosy prevalence was lower in villages compared to rural surrounding areas(Sommerfelt et al., 1985). These findings may be influenced by several factors. Urban areas have higher concentrations of healthcare facilities, which potentially aids in increased case detection, making the higher incidence rates an artifact of improved surveillance. Urban areas can also vary greatly in their conditions. The association with urban areas may be

more related to impoverished neighborhoods where water and sanitation measures may be lacking, and crowding may cause an increased risk of person-to-person transmission.

Proximity to water bodies has also been assessed as a risk factor. In Malawi, a cohort study reported decreasing leprosy incidence rates as the distance from a river or lake increased (Sterne, Pönnighaus, Fine, & Malema, 1995). In Bahia, Brazil, the relative risk for leprosy was reported as being higher in regions with greater inland water coverage (Cabral-Miranda et al., 2014). While the exact mechanisms behind this association are unclear, proximity to water bodies may be an environmental risk factor for leprosy.

Helminth Co-infections

Co-infections with helminths have also been studied as a leprosy risk factor. A spatial and temporal analysis of leprosy and schistosomiasis in Minas Gerais, Brazil found an increased relative risk of leprosy in areas with schistosomiasis (Phillips et al., 2017). The incidence remained significant when controlling for income and population density (Phillips et al., 2017). The incidence of leprosy was also found to have a positive relationship with schistosomiasis cases over time (Phillips et al., 2017).

Helminth co-infections have been associated with the more infectious forms of leprosy. In Burkina Faso, approximately twice the frequency of lepromatous leprosy was reported in an area endemic to onchocerciasis compared to an area with similar leprosy prevalence without onchocerciasis (Prost, Nebout, & Rougemont, 1979). Prevalence of multibacillary leprosy has similarly been found to be associated with helminth co-infections in Brazil (L. Diniz et al., 2002).

Helminths may play a role in the risk for leprosy reactions as well, however, the relationship is less clear. Type 2 reactions in leprosy patients have been reported to be associated

with helminth infections in Indonesia (Oktaria et al., 2016). However, a study in Nepal showed a negative relationship between Type 1 and Type 2 leprosy reactions and helminth co-infections, though Type 1 reactions made up a majority of those included in the study (Hagge et al., 2017). In Brazil, a recent case-control study found no association with helminth co-infections and leprosy reactions (Fairley et al., 2019). Whether this indicates a complex and differential relationship between helminth co-infections and types of leprosy reactions is unclear.

There is evidence that helminth infection can cause dysregulation in the immune system of the host which may provide a mechanism behind this association. Infection with helminths may result in an upregulation of the Th2 immune response, which would cause a downregulation in the Th1 mediated immune response responsible for protection against *M. leprae* (Chatterjee & Nutman, 2015). The Th1 and Th2 immune response both rely on the differentiation of CD4+ cells to produce crucial cytokines, interferon-gamma (IFN-γ) and interleukin 4 (IL-4), in mutually exclusive production pathways (Nath et al., 2015). In this relationship, increased levels of cytokines associated with one T helper immune response type are expected to be associated with decreased levels in the other. In leprosy patients, expression of Th1 cytokines has been shown to be associated with PB leprosy while patients with MB leprosy showed higher levels of Th2 cytokines, indicating that lower expression of Th1 cytokines is linked with increased bacillary counts and infectiousness associated with MB leprosy (Nath et al., 2015; Yamamura et al., 1991). An induced increase in levels of IFN-γ has additionally been shown to aid in rapid bacillary clearance in leprosy patients with lepromatous leprosy (Nath et al., 2015; L. H. Sampaio et al., 2011). Within the proposed model of helminth immune dysregulation, these findings are consistent with the association of helminth co-infections with MB and lepromatous forms of leprosy as seen in previous studies (L. Diniz et al., 2002; Prost et al., 1979).

The helminth-mediated immune dysregulation model has been suggested in tuberculosis, another mycobacterium disease related to leprosy where helminth co-infections may be a risk factor (Chatterjee & Nutman, 2015; Elias, Mengistu, Akuffo, & Britton, 2006). However, few studies have investigated this model in leprosy. A case-control study of leprosy patients and household contacts by Diniz et. al. reported Th2 cytokine expression to be higher in co-infected leprosy patients than leprosy patients without helminth co-infections (L. M. Diniz et al., 2010). Levels of IL-4 and IL-10 were significantly different between the groups, with IL-4 being about twice as concentrated in co-infected patients compared to patients without co-infections (L. M. Diniz et al., 2010). IL-4 and IL-10 are responsible for downregulating the Th1 pathway triggered in response to *M. leprae* and therefore decreasing the immune response of the host (Krutzik et al., 2003; Nath et al., 2015; Pinheiro et al., 2018). Diniz et. al. also found evidence of downregulation of the Th1 pathway (L. M. Diniz et al., 2010). Levels of intracellular IFN-γ in co-infected patients were found to be about half that found in leprosy patients without co-infections (L. M. Diniz et al., 2010). IFN-γ is known to be heavily associated with the Th1 immune response (Yamamura et al., 1991). Patients with higher bacillary counts, such as in patients with lepromatous leprosy, were found to have particularly low IFN-γ (L. M. Diniz et al., 2010). Unfortunately, co-infected group counts were low, particularly when stratified by forms of leprosy, which may influence the results (L. M. Diniz et al., 2010). More research is needed to confirm these findings, and improve the understanding of helminths as a risk factor for leprosy.

An additional area that warrants further study is the role of the T helper Type 17 (Th17) cell-mediated immune response in leprosy and helminth co-infections. Th17 is a relatively new type of inflammatory immune response found in humans (Bettelli, Korn, Oukka, & Kuchroo, 2008). The Th17 immune is proposed to be associated with inflammatory responses and the clearance of

extracellular pathogens (Bettelli et al., 2008). The signature cytokine of the Th17 immune response, interleukin 17 (IL 17), has been reported to promote Th1 cytokines such as tumor necrosis factor-alpha (TNF-α), and increase neutrophil recruitment (Bettelli et al., 2008; Nath et al., 2015; Sadhu et al., 2016). The Th17 immune response has been studied in leishmaniosis and helminth infections, tuberculosis, and leprosy.

The role of IL 17 in the immune response to helminths and other parasitic diseases is unclear. A study in patients with leishmaniosis found that IL-17 expression was associated with an overexcited inflammatory response to the infection (Gonzalez-Lombana et al., 2013). However, IL-17 levels appeared to be regulated by levels of both Th1 and Th2 cytokines. IL-17 levels were higher when Th2 cytokine IL-10 levels were low, however IL-17 levels were also increased when Th1 cytokine IL-10 was blocked (Gonzalez-Lombana et al., 2013). In a study on intestinal helminth *Heligmosomoides polygyrus* in mice, helminth infection was found to decrease detected levels IL-17 in lymph nodes and IL-17 production (Elliott et al., 2008). A study in *Schistosoma mansoni* infection in mice found that helminth infection resulted in IL-10 regulation of IL-17, however IL-17 expression increased in mice with both an *S. mansoni* and bacterial infection (Perona-Wright et al., 2012). This finding is potentially interesting for its implications for mycobacterium and helminth coinfections.

In tuberculosis, IL-17 has also been associated with a strong inflammatory immune response to infection, and there is additional evidence for the regulatory effects of IL-10 (Torrado & Cooper, 2010). In a study of tuberculosis patients with filarial co-infections, expression of both Th1 and Th17 immune responses were decreased in co-infected patients (Babu et al., 2009).

In leprosy patients in New Delhi, Sadhu et al. reported higher levels of Il-17 detected in patients with tuberculoid forms of leprosy compared to patients with lepromatous forms (Sadhu

et al., 2016). This was found to be highly mediated by levels of inhibiting IL-10 cytokines. When IL-10 cytokines were blocked, an increased level of IL-17 expression was seen (Sadhu et al., 2016). As helminth co-infections are suspected to upregulate the Th2 immune cytokines, these findings show that removal of helminth infections from leprosy patients may enhance the patient's innate immune response to the disease (Sadhu et al., 2016). The Th17 immune response is, therefore, a particular area of interest in leprosy research.

In this study, we examine the relationship between schistosomiasis co-infection and levels of cytokines associated with the innate immune response in *M. leprae* in Minas Gerais, Brazil. Minas Gerais is endemic to both schistosomiasis and leprosy, and spatial associations between the two diseases have been reported in this state (Phillips et al., 2017). This study is one of the few to look at schistosomiasis as a risk factor for leprosy and adds to the limited research on the role of helminth co-infections in leprosy risk. This study will help support previous findings on the role of immune dysregulation associated with helminth infection in leprosy patients. Levels of IFN-γ, TNF-α, IL-10, and IL-4 were assessed consistent with previous research. Levels of IL-17 were additionally assessed. Comparisons were made between leprosy patients with and without schistosomiasis co-infection and healthy controls with and without schistosomiasis. This study will delineate the role of schistosomiasis in leprosy immune response, which hasn't previously been done in this area. Stool samples are additionally used instead of serology to assess helminth infection, limiting the analysis to current co-infections.

III. Manuscript

The Impact of Schistosomiasis Co-infections on Immune Responses in Leprosy

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Abstract

Background: Leprosy is an ancient neglected tropical disease caused by *Mycobacterium leprae*. India and Brazil carry the highest burden of disease, much of which is concentrated in hyperendemic areas with more than 40 new cases per 100,000 people annually. Helminth co-infections, like schistosomiasis, can cause immune dysregulation and may contribute to disease transmission.

Methods: A case-control study was performed in the state of Minas Gerais, Brazil. Participants were recruited from the communities surrounding the city of Governador Valadares. A total of 260 participants were recruited, 79 leprosy patients, and 178 healthy adults. Information on lifestyle factors was collected via questionnaires. Stool and blood samples were collected and tested for schistosomiasis and cytokine levels, respectively. Schistosomiasis was diagnosed using Keto Katz methods. Levels of Th1 cytokines IFN-γ and TNF-α, Th2 cytokines IL-4 and IL-10, and Th17 cytokine IL-17 were assessed using flow cytometry with the BD ™ Cytometric Bead Array system stimulated with *M. leprae*. Descriptive, one-way ANOVA and ANCOVA analyses were performed to compare across infections.

Results: Significant differences were detected in levels of IFN-γ ($F=3.18$, $p=0.03$), TNF-α ($F=6.20$, $p<0.001$), between the four groups when controlling for age, sex, and vitamin D deficiency. IFN-γ was found to be decreased in leprosy patients with schistosomiasis co-infections. TNF-α, conversely, was found to be significantly increased in leprosy patients with schistosomiasis co-infections. Levels of IL-10 were found to be higher in leprosy patients with schistosomiasis co-infections, however, the differences were not found to be significant ($F=2.05$, $p=0.11$). Additionally, average levels of IL-17 were found to be lower in individuals with schistosomiasis co-infections, however, these were also found to be insignificantly different from

the other groups (F=0.93, p=0.43). No significant or perceived differences were found in IL-4 levels between groups (F=1.53, p= 0.21).

Conclusion: These results support the theory that schistosomiasis co-infections upregulate the Th2 immune response, and downregulate the protective Th1 immune response to *M. leprae*, potentially making individuals more susceptible to disease. These findings suggest individuals living in areas endemic for schistosomiasis and leprosy may be at a higher risk for leprosy and open up the potential for new avenues to be explored in transmission control efforts.

Introduction

Leprosy is an ancient neglected tropical disease caused by a bacterial infection of the skin and peripheral nerves by *Mycobacterium leprae (M. leprae)* (White & Franco-Paredes, 2015). While the disease is typically not fatal, it can lead to permanent nerve damage, disability, and disfigurement if left untreated (WHO, 2017). While global disease prevalence has decreased due to the availability of a multidrug therapy, incidence rates persist (Rodrigues & Lockwood, 2011). In 2018, over 200,000 new cases were reported globally (WHO, 2019a). Brazil is among those with the highest burden of disease, accounting for the second highest concentration of incident cases in the world, and 93% of those in the Americas as of 2018 (WHO, 2019). While improved case detection and diagnosis are areas requiring improvement, any effort towards decreasing leprosy incidence rates will require a greater understanding of leprosy transmission and risk factors.

M. leprae is an acid-fast mycobacterium, primarily transmitted via respiratory droplets. However, it is estimated that only 5-10% of people exposed to leprosy develop the disease (Lockwood & Saunderson, 2012; Rodrigues & Lockwood, 2011; White & Franco-Paredes,

2015). What makes those 5-10% of people particularly vulnerable is poorly understood. Many of the leprosy incident cases are concentrated in hyperendemic areas with more than 40 new cases per 100,000 persons each year. The presence of these geographic clusters suggests that there are environmental or socio-contextual factors influencing the spread of disease. WASH factors, poor and crowded living conditions, and nutrition have been investigated as potential factors influencing transmission (Cabral-Miranda, Chiaravalloti Neto, & Barrozo, 2014; Wagenaar et al., 2015). Co-infections with helminths have also been investigated as a potential factor influencing disease transmission (Diniz, Magalhaes, Pereira, Dietze, & Ribeiro-Rodrigues, 2010; Hagge et al., 2017; Phillips et al., 2017).

In the southeastern state of Minas Gerais, an endemic state in Brazil, reports of schistosomiasis were spatially correlated with areas of high leprosy incidence and associated with cases of the most infectious form of leprosy (Phillips et al., 2017). In Espirito Santo, Brazil, helminth co-infections were similarly associated with the more severe and infectious forms of leprosy (Diniz et al., 2010). It is hypothesized that helminth infections may interfere with the host immune response to *M. leprae* leading to greater susceptibility to more severe infections. Leprosy patients with helminth co-infections have been found to have disrupted immune responses away from cell-mediated immunity and higher levels of cytokines responsible for downregulating the immune response to *M. leprae* (Diniz et al., 2010; Hagge et al., 2017). These findings present helminth co-infections as an intriguing opportunity to investigate the potential mechanisms influencing leprosy transmission and hyperendemic areas. In this study, we investigated the effect of schistosomiasis co-infections on levels of cytokines associated with the innate immune response to *M. leprae*. in Minas Gerais, Brazil, accounting for Vitamin D deficiencies, age, and sex.

Methods

Study Population

The case-control study was performed in the Governador Valadares and Mantena municipalities the state of Minas Gerais, Brazil, an area with high incidence rates for leprosy and schistosomiasis infections (Phillips et al., 2017). The study team included researchers from Emory University, Atlanta, GA, Universidad Federal de Juiz de For, and Universidad Vale do Rio Doce. Participants were recruited from a leprosy referral clinic in the city of Governador Valadares, and at family health clinics in the rural communities surrounding the city from June 2016 to December 2018.

Participants were enrolled in one of three groups, leprosy cases, healthy controls, and household contacts. All participants were over the age of 3 and no pregnant women were recruited. Patients were eligible to enroll if they were diagnosed with leprosy within the previous 30 days and had no history of treatment for leprosy. Healthy controls were included if they had no current or prior diagnosis of leprosy and excluded if they had a history of undiagnosed skin or nerve disorder, or gastrointestinal symptoms and weight loss. Close household contacts were recruited as a second control group if they fit the criteria for healthy controls and lived in the same household or had regular daily contact with the index patient. Non-contact controls were matched with cases by age (within 5 years older or younger), sex, and community of origin to the extent that it was possible.

Data Collection

After the informed consent process was completed, a study team dermatologist conducted physical examinations of participants and took skin smears. Leprosy was diagnosed in accordance with the WHO guidelines and using the Madrid classification (Congress, 1953). Skin

smears were analyzed to determine the clinical pathologic type of leprosy infection. A questionnaire was administered to all groups and information on demographics, socioeconomic status, residence, and nutrition of participants was collected. Additional demographic and clinical information was obtained from medical records. A blood sample was also collected in all groups by a trained study nurse and used to test of micronutrients, blood count, and cytokine profiles. Cytokines specific to the Th1, Th2, and Th17 immune responses were assessed. Levels IFN-γ, TNF-α, IL-10, IL-4, and IL-17 were assessed in supernatant samples of peripheral blood mononuclear cells (PBMC) using flow cytometry with the BD ™ Cytometric Bead Array (CBA) Human Th1/Th2/Th17 Kit after stimulation with *M. leprae*. After a 3-hour incubation at room temperature, capture spheres were washed and centrifuged for 7 minutes at 18°C. The CBA capture spheres were then analyzed using BD FACS Verse ™. Stool samples were collected from each participant for 3 days, in accordance with standard practice, and analyzed for parasites and helminth ova to diagnose helminth infections using Kata Katz and HPJ methods. The presence of eggs in any of the samples was defined as a positive diagnosis of helminth infection. Vitamin D deficiency was defined by 25-OH vitamin D concentrations below 20 μg/dL.

Data Analysis

The sample size for this study was determined from the prevalence of helminth co-infections in leprosy patients previously reported by Diniz et. al. (Diniz et al., 2010). Diniz et al. reported leprosy cases having 4.0 the odds of helminth infection compared to healthy controls. When comparing multibacillary and paucibacillary forms of leprosy, the odds ratio for helminth infections was found to be 6.24. An 80% power was used with a two-sided confidence level of 95%. To be powered to detect differences between forms of leprosy 31 patients would need to be included for each form of leprosy (multibacillary and paucibacillary), for a total of 62 cases and

62 per control group. The dropout rate for this study was expected to be 10%. A total population of 207 participants was calculated, which was rounded up to 210 participants with 70 participants per group.

Associations between schistosomiasis infection and leprosy were assessed using chi-squared analyses. Participants were grouped by leprosy and schistosomiasis infection status. One-way ANOVA was performed to assess differences in cytokine levels between the groups. Cytokine levels were found to be abnormally distributed and were log-transformed to meet the assumptions of normality. Scheffé's test was used to assess pairwise comparisons due to unequal sample sizes in the groups. ANCOVA analyses were performed to account for the effect of covariates sex, age, as well as vitamin D deficiency. Vitamin D and has been found to be involved in the Th1 immune response, and be expressed differently in tuberculoid and lepromatous forms of leprosy (Nath et al., 2015). The healthy control and household contact groups were combined for the purposes of these analyses. All statistical analyses were performed in SAS 9.4.

Ethical Considerations

Ethical approvals were obtained from the Emory University and Universidade Federal de Juiz de Fora Institutional Review Boards.

Results

A total of 256 individuals were enrolled in the study, including 79 leprosy patients, and 177 healthy subjects. Demographic characteristics were similar when compared between leprosy patients and healthy participants (Table 1). The median age for leprosy patients was 47, ranging from 6 to 80 years old. This was higher than the median age for healthy controls and household contacts at 38 years with a range of 5-85 years. Each group had an approximately equal

distribution of males and females. Of the leprosy patients, 55.7% were men compared to 44.6% of healthy participants. Most participants in both groups identified as mixed-race, with white, and black being the next most common racial identity. Of the leprosy patients enrolled in the study, 58.2% identified as mixed race, 20.9% as black, and 19.4% as white. A similar 57.1% of healthy participants self-identified as multi-racial, with 25.9% identifying as white, and 15.3% identifying as black. Education levels and salary were also assessed as proxies for socioeconomic status. Most participants had a primary school level education in both categories, at 70.9% of leprosy patients and 57.6% of healthy participants. Tertiary education was acquired by only 1% of leprosy patients, and 8% of healthy controls. Leprosy patients reported having no education slightly more than healthy participants at about 15% compared to about 9% of healthy participants. Less than minimum wage salaries were prevalent, with 38.5% of leprosy patients and 28.4% of healthy participants giving this response. One to three times the minimum wage was the most common response, reported in 53.9% of leprosy patients and 62.5% of healthy participants. Less than 10% of participants in both groups had 3-5 times the minimum wage. Only 1-2% of participants in both groups had salaries over 5 times the minimum wage. A larger proportion of leprosy patients lived in rural areas at 48.7% compared to 40.9% of healthy participants. Vitamin D deficiency was found to be about twice as prevalent in leprosy patients at 20.8% compared to 9.8% of healthy participants.

Stool samples for schistosomiasis tests were available for 241 participants. A total of 16 participants tested positive, 8 (4.8%) healthy controls, and 8 (10.7%) leprosy patients. Fifteen participants did not submit a stool sample, however, these were approximately equal between groups, representing 6.2% of healthy participants and 5.1% of leprosy patients. A Chi-squared analysis showed the odds of having schistosomiasis for leprosy patients were at 2.36 times the

odds for healthy participants, however these results were statistically insignificant (p=0.09; 95%CI = 0.85, 6.54).

Distribution of cytokines TNF-α, IFN-γ, IL-4, IL-10, and IL-17 are shown in Figure 1 with results of ANCOVA analyses listed in Tables 2-6. Statistically significant differences were found in log-transformed levels of IFN-γ between the four groups (F= 3.18, p=0.02). Mean log-transformed levels of IFN-γ were found to be significantly lower in leprosy patients with schistosomiasis co-infections compared to healthy participants without schistosomiasis co-infections by -2.01 units (95% CI: -3.99, -0.21). This relationship remained consistent when controlling for the effects of age, sex, and vitamin D deficiency. Sex was the only covariate to be statistically significant in these analyses. Decreased mean levels of IFN-γ were also seen between leprosy patients with schistosomiasis co-infections (4.33±2.31) compared to those without (6.17±1.77), as well as healthy participants with schistosomiasis infections (5.43±2.17) compared to healthy participants without schistosomiasis infections (6.34±1.78). However, these relationships weren't found to be statistically significant.

Levels of TNF-α were found to be significantly different between groups (F= 6.20, p<0.001). Average log TNF-α levels were higher in leprosy patients without schistosomiasis co-infections compared to healthy participants without schistosomiasis co-infections by 0.83 (95% CI: 0.14, 1.51). This relationship was enhanced when accounting for sex and age (F=6.61, p<0.001), but weakened when controlling for vitamin D deficiency (F=5.21, p=0.002). Mean log TNF-α levels also appeared elevated in healthy participants with schistosomiasis co-infections (3.05±1.22) compared to without schistosomiasis co-infections (1.91± 1.44), and leprosy patients with schistosomiasis infections (3.35±1.35), and those without (1.74±1.71).

Mean levels of IL-4 were consistent across all groups with no significant or perceivable differences (F= 1.53, p=0.21). This result was only weakened when controlling for age, and vitamin d deficiency (Table 4; F=0.93, p=0.43). While controlling for sex increased the strength of the differences, the results remained statistically insignificant (F=2.56, p=0.20). Mean levels of IL-10 were not significantly different between groups (F=2.05, p=0.12). A higher mean log IL-10 level in leprosy patients with schistosomiasis co-infections (4.53±0.88), compared to leprosy patients without schistosomiasis co-infections (3.52 ±1.48) could be perceived, however the results were statistically insignificant. Controlling for age and sex increased the strength of the variance, but it remained statistically insignificant (F=2.14, p=0.10). Controlling for vitamin D only decreased the strength of the results (F=1.75, p=0.16).

Mean levels of IL-17 were found to be insignificantly different between groups (F=0.93, p=0.43). However, lower levels were seen in leprosy patients with schistosomiasis co-infections (1.08±1.10) compared to leprosy patients without (2.04±1.39), though the difference was not significant. Mean log IL-17 levels appeared consistent in healthy participants with (1.75±0.56) and without schistosomiasis infections (1.94±1.37). Controlling for age and sex significantly decreased the strength of these differences in ANCOVA analyses (F=0.47, p=0.70). Controlling for vitamin D deficiencies only mildly strengthened the results (F=1.07, p=0.36).

Discussion

Our study found significant and perceived differences in levels of IFN-γ, TNF-α, IL-10 and IL-17 between the four groups. IFN-γ was shown to be decreased in leprosy patients with schistosomiasis co-infections. The lower levels of IFN-γ are consistent with previous findings in leprosy patients with helminth co-infections (L. M. Diniz et al., 2010). However, only differences in log IFN-γ levels between non-infected healthy participants and co-infected leprosy

patients were found to be significant in these data. The decreased levels of IFN-γ seen in this study are consistent with a downregulated Th1 immune response, as IFN-γ is heavily associated with that pathway (Yamamura et al., 1991).

This study also supports an elevated Th2 immune expression in participants with schistosomiasis co-infections. Levels of IL-10 were found to be higher in leprosy patients with schistosomiasis co-infections. Increased levels of IL-10 have previously been reported in leprosy patients with helminth co-infections (L. M. Diniz et al., 2010). However, no perceived difference was found in IL-4 levels between groups. The lack of a difference in IL-4 levels between groups is inconsistent with previous findings showing an increase in IL-4 in participants with helminth co-infections (Diniz et al., 2010). IL-4 is also associated with the Th2 immune response, and an increase in this biomarker would be expected in co-infected individuals (Diniz et al., 2010).

Evidence of differences in Th17 expression between groups was also found in this study. Average log IL-17 levels were found to be lower in individuals with schistosomiasis co-infections. This has been previously reported in tuberculosis patients with filarial co-infections (Babu et al., 2009). IL-17 cytokine expression has been shown to be regulated by IL-10 expression in leprosy patients, consistent with downregulation of the Th17 immune response when the Th2 immune response is upregulated (Sadhu et al., 2016). The decreased levels of IL-17 and elevated levels of IL-10 seen in this study are also congruent with this theory, as well as the impact of helminth coinfections on the inflammatory immune response.

The elevated log TNF-α levels seen in leprosy patients compared to healthy participants with no infection is consistent with previous findings showing elevated TNF-α levels in leprosy patients (Parida, Grau, Zaheer, & Mukherjee, 1992). However, the elevated levels of leprosy patients with schistosomiasis co-infections perceived in the data compared to leprosy patients

without schistosomiasis co-infections was unexpected. TNF-α is typically associated with the Th1 immune response; however, the relationship is complicated. These results highlight the complexity of the role of TNF-α in helminth leprosy co-infections, and a need to further investigate this inflammatory biomarker in leprosy. One possible explanation for this finding may exist in previous research on leprosy reactions, a condition characterized by a sudden increase in severity of the disease during treatment (Parida et al., 1992). Previous studies have shown TNF-α to be elevated in leprosy reactions, however the precise role of the cytokine is still being investigated (Parida et al., 1992). The impact of helminth co-infections on the prevalence of leprosy reactions has also been previously studied, however findings are mixed (Hagge et al., 2017; Oktaria et al., 2016). One study reported elevated rates of Type II leprosy reactions in individuals with helminth co-infections in Indonesia (Oktaria et al., 2016). The findings for TNF-α in this study may be further complicated by the impact of variation in cytokine expression in different forms of leprosy. Clinical forms of leprosy were combined in these analyses, so differences in TNF-α expression wasn't assessed. However, previous literature has shown a variation in cytokine expression by clinical form with Th1 cytokines being associated with PB leprosy and Th2 cytokine expression being associated with MB leprosy (Yamamura et al., 1991). Although further research is needed to fully understand this relationship, the elevated levels of TNF-α seen in leprosy patients with schistosomiasis co-infections may highlight a potential mechanism behind the association of helminth-coinfections and leprosy reactions.

This study is one of only a few studies to look at cytokine levels in leprosy patients co-infected in helminths in Brazil. This is an area where further research has been needed, particularly in areas endemic for leprosy and schistosomiasis. This study is also one the few to look at a single genus of helminth co-infections, and schistosomiasis in particular.

Schistosomiasis is highly prevalent in the area, making it of interest in investigating leprosy immune responses as leprosy is also hyperendemic in the region (Phillips et al., 2017). Finally, this study had high enrollment relative to previous literature on this topic with 260 individuals enrolled.

There are several limitations to this study. The number of confirmed current schistosomiasis infections was relatively low in our study population, but consistent with previous literature (L. M. Diniz et al., 2010). The lack of more information on poverty measures and living conditions is another limitation, as these are variables of interest in understanding leprosy transmission (Cabral-Miranda, Chiaravalloti Neto, & Barrozo, 2014). Crowding in particular has been hypothesized to impact leprosy transmission and wasn't captured by our data collection instrument (Cabral-Miranda et al., 2014). Several participants had missing or incomplete data, including failing to provide a stool sample for schistosomiasis diagnosis. Additionally, this study did not achieve power to stratify by clinical form of leprosy. No assessments were possible on associations between schistosomiasis co-infection and the polar ends of the leprosy spectrum like tuberculoid vs lepromatous leprosy, as has been previously reported, due to small sample sizes after stratification (L. M. Diniz et al., 2010). Additionally, combining these clinical forms into a single group may have impacted our analyses, as cytokine expression varies between forms (Yamamura et al., 1991). Very few leprosy patients were recorded to have reactions in this population, so no assessment of the association between helminth infection and reaction prevalence could be conducted. Age, sex, and vitamin D levels were the only variables controlled for in these analyses, additional analyses of the relationship between schistosomiasis infections and cytokine levels in this study population, controlling for other potentially confounding variables, are warranted.

This study provides support for the theory that schistosomiasis co-infections dysregulate the immune response to *M. leprae* through upregulation of the Th2 immune response and coinciding downregulation of the protective Th1 response. These preliminary findings combined with other evidence linking helminth coinfections with leprosy transmission, provide a potential explanation to the presence of hyperendemic areas in regions co-endemic for leprosy and schistosomiasis. A downregulated Th1 response has been associated MB leprosy, the more infectious form of the disease (Nath et al., 2015; Yamamura et al., 1991). Populations living in areas endemic to schistosomiasis may, therefore, be both more susceptible to contracting leprosy, as well as increased leprosy transmission rates, further increasing the population incidence rates.

These findings may also provide a biological mechanism behind the link between leprosy cases and poverty, as schistosomiasis infection is also heavily associated with living in impoverished conditions (WHO, n.d.). The evidence of schistosomiasis co-infection as a risk factor for leprosy transmission has the potential to open up alternative avenues to decrease leprosy incidence rates around the world. Previous research has shown that blocking Th2 cytokine expression results in increased expression of the Th17 immune response, which in turn promotes the Th1 immune response (Bettelli et al., 2008; Sadhu et al., 2016). Treating co-infected leprosy patients for helminth infections may, therefore, result in a recovery of the patient's innate immune response to leprosy. These findings also indicate the possibility of collaboration between helminth and leprosy control efforts, which may aid in decreasing the global burden of both diseases. Further studies are needed to improve our understanding of the mechanisms behind the dysregulation of *M. leprae* immune responses by schistosomiasis and identify potential opportunities for intervention.

IV. Tables and Figures

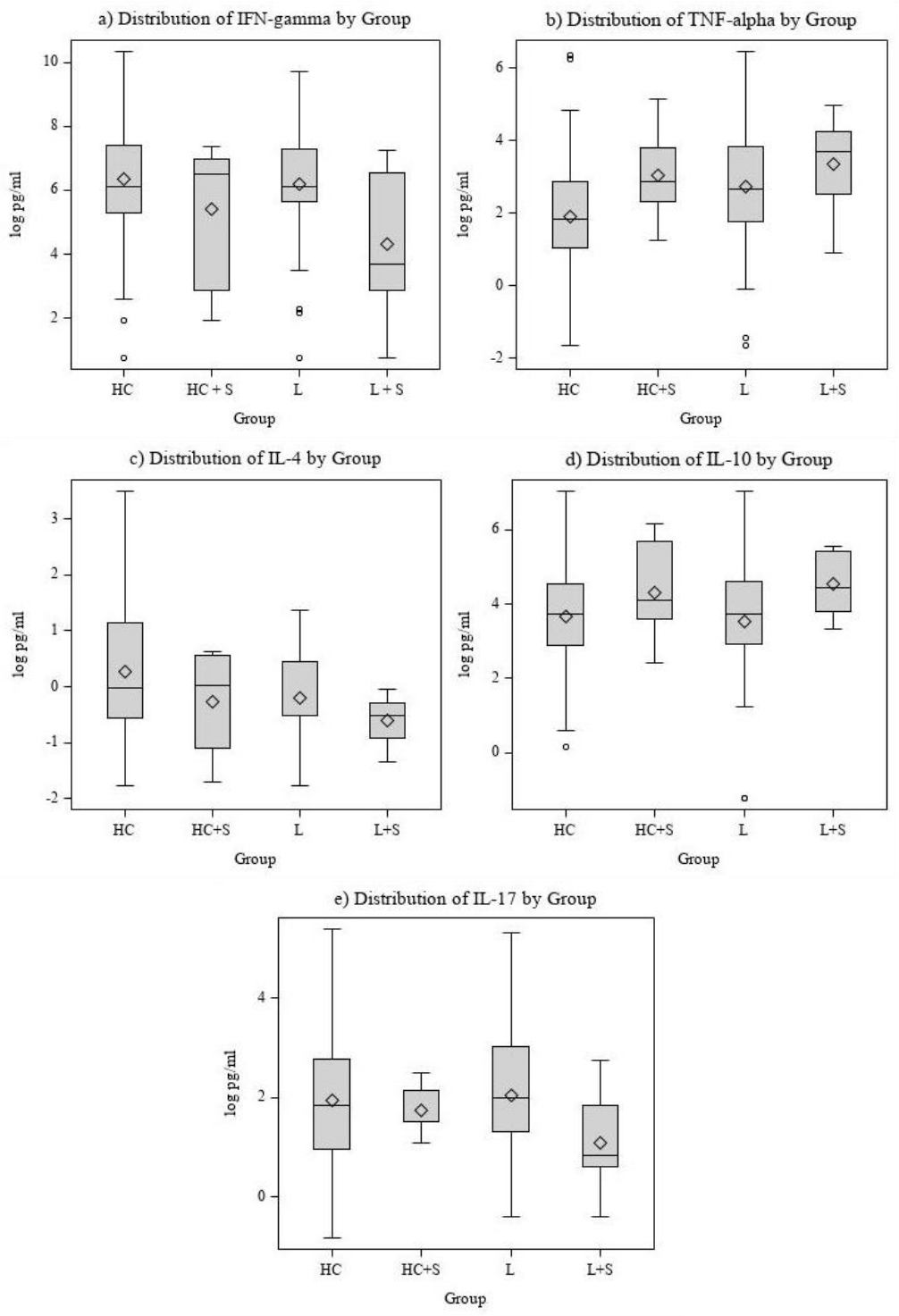
Table 1. Population characteristics for both leprosy patients and healthy controls.

Characteristics*	Leprosy Patients (n= 79)	Healthy Controls (n= 177)
Age (years)**	47.00 (6-80)	38 (5-85)
Sex		
Male	44 (55.70)	79 (44.63)
Race		
White	13 (19.40)	44 (25.88)
Black	14 (20.90)	26 (15.29)
Asian	0 (0.00)	3 (1.76)
Multi-racial	39 (58.21)	97 (57.06)
Indigenous	1 (1.49)	0 (0.00)
Education Level		
Tertiary Education	1 (1.27)	14 (7.91)
Secondary Education	11 (13.92)	43 (24.29)
Primary Education	56 (70.89)	102 (57.63)
No Education	11 (13.92)	16 (9.04)
Salary		
Less than minimum wage	30 (38.46)	50 (28.41)
1-3 times minimum wage	42 (53.85)	110 (62.50)
3-5 times minimum wage	4 (5.13)	11 (6.25)
> 5 times minimum wage	1(1.28)	2 (1.14)
Urbanity		
Urban	38 (51.35)	104 (59.09)
Rural	36 (48.65)	72 (40.91)
Schistosomiasis	8 (10.67)	8 (4.82)
Vitamin D Deficiency	15 (20.83)	16 (9.82)

*Given in n (%).

**Given in median (range).

Figure 1. Distribution of log transformed levels of IFN-γ, TNF-α, IL-10, IL-4 and IL-17 in leprosy patients and healthy participants with and without schistosomiasis infection.



Cytokine levels were compared across healthy controls and household contacts without helminth infections (HC), healthy controls and household contacts with helminth infections (HC+S), leprosy patients without helminth co-infections (L), and co-infected leprosy patients (L+S).

Table 2. Analysis of Covariance (ANCOVA) of group differences in IFN-γ distribution controlling for age, sex, and vitamin D deficiency

Covariate	df	F	p-value
None	3	3.18	0.025*
Sex	4	2.75	0.044*
Age	4	3.01	0.032*
Vitamin D deficiency	4	3.66	0.014*
Age x Sex	5	2.47	0.064*
Age x Vitamin D deficiency	5	3.30	0.022*
Sex x Vitamin D deficiency	5	3.46	0.018*
Age x Sex x Vitamin D deficiency	6	3.00	0.032*

* $p < 0.05$

Table 3. Analysis of Covariance (ANCOVA) of group differences in TNF-α distribution controlling for age, sex, and vitamin D deficiency

Covariate	df	F	p-value
None	3	6.20	<0.001**
Sex	4	6.44	<0.001**
Age	4	6.41	<0.001**
Vitamin D deficiency	4	5.21	0.002**
Age x Sex	5	6.61	<0.001**
Age x Vitamin D deficiency	5	5.62	0.001**
Sex x Vitamin D deficiency	5	5.38	0.001**
Age x Sex x Vitamin D deficiency	6	5.76	<0.001**

* $p < 0.05$, ** $p < 0.01$

Table 4. Analysis of Covariance (ANCOVA) of group differences in IL-4 distribution controlling for age, sex, and vitamin D deficiency

Covariate	df	F	p-value
None	3	1.53	0.212
Sex	4	2.56	0.203
Age	4	1.50	0.219
Vitamin D deficiency	4	0.94	0.426
Age x Sex	5	1.53	0.212
Age x Vitamin D deficiency	5	0.93	0.432
Sex x Vitamin D deficiency	5	0.97	0.410
Age x Sex x Vitamin D deficiency	6	0.95	0.421

Table 5. Analysis of Covariance (ANCOVA) of group differences in IL-10 distribution controlling for age, sex, and vitamin D deficiency

Covariate	df	F	p-value
None	3	2.05	0.108
Sex	4	2.11	0.101
Age	4	2.03	0.111
Vitamin D deficiency	4	1.75	0.159
Age x Sex	5	2.14	0.097
Age x Vitamin D deficiency	5	1.84	0.142
Sex x Vitamin D deficiency	5	1.73	0.163
Age x Sex x Vitamin D deficiency	6	1.87	0.135

Table 6. Analysis of Covariance (ANCOVA) of group differences in IL-17 distribution controlling for age, sex, and vitamin D deficiency

Covariate	df	F	p-value
None	3	0.93	0.428
Sex	4	0.86	0.462
Age	4	0.54	0.655
Vitamin D deficiency	4	1.07	0.363
Age x Sex	5	0.47	0.704
Age x Vitamin D deficiency	5	0.60	0.614
Sex x Vitamin D deficiency	5	1.05	0.375
Age x Sex x Vitamin D deficiency	6	0.57	0.635

V. Implications and Recommendations

This study found that levels of cytokines associated with Th1 and Th2 immune responses were significantly different between leprosy patients with and without schistosomiasis infections compared to healthy controls with and without schistosomiasis infections. Levels of TNF-α were significantly different between the four groups, even after controlling for sex, age, and vitamin D deficiency. Levels of IFN-γ were also found to be significantly different between the four groups, which remained after controlling for sex, age, and vitamin D deficiency. Levels of IL-17, IL-4, and IL-10 were not significantly different across groups.

These findings are consistent with the model of helminth mediated immune dysregulation and previous literature (L. M. Diniz et al., 2010). Decreased levels of IFN-γ observed in leprosy patients with helminth co-infections are consistent with the suppression of the Th1 immune response and prior literature (Chatterjee & Nutman, 2015; L. M. Diniz et al., 2010).

This study provides further evidence to support infection with helminths as a risk factor for leprosy. This association may help provide a mechanism behind other environmental and sociocontextual determinants of leprosy identified in prior literature that may also be associated with helminth infection such as poverty, and poor sanitation (Castro et al., 2016; Chaves et al., 2017; Duarte-Cunha et al., 2015; Freitas & P. Garcia, 2014; Kerr-Pontes et al., 2004; Murto et al., 2013; Nobre et al., 2015). Hyperendemic areas for leprosy have already been shown to be correlated with schistosomiasis endemic areas (Phillips et al., 2017). These findings support evidence that immune dysregulation due to schistosomiasis infection may play a role in the formation of these high leprosy incidence areas (L. M. Diniz et al., 2010). Immune dysregulation caused by schistosomiasis may also help explain how some people seem to be far more

vulnerable to leprosy, with only 5-10% of people exposed contracting the disease (Rodrigues & Lockwood, 2011).

Establishing helminths as a risk factor for leprosy also has the potential to aid in leprosy elimination efforts. In 2016, the World Health Organization launched its most recent leprosy elimination campaign the Global Leprosy Strategy 2016-2020 (WHO, 2019a). Targets of the intervention include zero prevalence of disabilities in pediatric leprosy incident cases and a grade-2 disability rate below 1 in 1 million people (WHO, 2019a). The strategy calls for several interventions including promoting early case detection, targeting detection in higher-risk groups, and increasing health care coverage in low resource populations (WHO, 2019a). While some global progress has been made toward these goals, Brazil has actually seen a mild increase across some of these measures. In 2018, 11,323 new leprosy cases with grade-2 disability were reported globally, a decrease from 2016 counts of 13,042 cases (WHO, 2019c). However, Brazil reported 2,109 cases in 2018 which was an increase from the 1,736 cases reported in 2016 (WHO, 2019c). Global leprosy incidence in children was reduced to 16,013 cases in 2018 from 18,475 in 2016 (WHO, 2019c). However, Brazil saw an increase in 2018 at 1705 cases compared to 1696 cases in 2016 (WHO, 2019c). While these trends may be in part due to increased surveillance efforts in Brazil as a result of the intervention, the targets of the Global Leprosy Strategy are far from being met.

Incorporating schistosomiasis as a risk factor into the leprosy elimination campaign may promote progress toward meeting the reported targets. The establishment of schistosomiasis infection as a risk factor for leprosy can help to target early case detection. Detecting cases early allows patients to start treatment earlier in the disease progression, limiting person-to-person transmission, and preventing disability. Populations with high rates of infection from

schistosomiasis and helminths should be considered at higher risk for leprosy in endemic countries. Targeting surveillance efforts in these regions may help identify cases earlier.

Efforts to control or eliminate schistosomiasis in Brazil may also help prevent incidence of leprosy and leprosy associated grade-2 disability. Eliminating schistosomiasis from hyperendemic areas may decrease leprosy risk in the exposed population. Additionally, elimination of schistosomiasis may decrease rates of grade-2 disability. Leprosy reactions are significantly associated with grade-2 disability (Raposo et al., 2018). The findings in this and previous studies supporting an association between leprosy reactions and schistosomiasis co-infection suggest that controlling schistosomiasis may aid in efforts to decrease grade-2 disability rates (Oktaria et al., 2016).

Finally, the association of increased leprosy risk and schistosomiasis infection provides an opportunity to increase resources for control efforts for both diseases. Both leprosy and schistosomiasis are classified as neglected tropical diseases, with relatively few resources. Utilizing surveillance efforts for schistosomiasis to supplement current leprosy case detection strategies may aid in early detection. Additionally, testing for schistosomiasis may be incorporated into leprosy diagnostic visits to aid in elimination.

More research is needed to further investigate the impact of schistosomiasis co-infection on the host immune response to *M. leprae*. The relationship has the potential to support novel approaches to leprosy elimination efforts, and clarify the role of environmental risk factors in leprosy incidence. The findings presented in this study were consistent with previous literature supporting a downregulation of Th1 immune response in leprosy patients with schistosomiasis co-infections (L. M. Diniz et al., 2010). Like previous studies, the findings were significantly

limited by low sample size after stratification. Larger scale studies are needed to clarify this relationship.

VI. References

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