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The Burden of Helminth Coinfections and Micronutrient Deficiencies in Patients with and without Leprosy Reactions: A Pilot Study in Minas Gerais, Brazil

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Abstract. Leprosy reactions are immune-mediated complications occurring in up to 50% of patients. The immune consequences of helminth infections and micronutrient deficiencies suggest a potential role in type 1 reactions (T1R) or type 2 reactions (T2R). We conducted a case–control study in Minas Gerais, Brazil, to evaluate whether comorbidities and other factors are associated with reactions in patients with multibacillary leprosy. Stool and serum were tested for helminth infections. Deficiencies of vitamin A, D, and iron were measured using serum retinol, 25-hydroxyvitamin D, and ferritin, respectively. Logistic regression models identified associations between reactions and helminth infections, micronutrient deficiencies, and other variables. Seventy-three patients were enrolled, 24 (33%) with T1R, 21 (29%) with T2R, 8 (15%) with mixed T1R/T2R, and 20 (27%) without reactions. Evidence of helminth infections were found in 11 participants (15%) and included IgG4 reactivity against *Schistosoma mansoni*, *Strongyloides*, and *Ascaris* antigens. Thirty-eight (52%) had vitamin D deficiency, eight (11%) had vitamin A insufficiency, 21 (29%) had anemia, and one (1.4%) had iron deficiency. Multivariable logistic regression showed no statistically significant associations between helminth coinfections and total reactions (adjusted odds ratios [aOR]: 1.36, 95% CI: 0.22, 8.33), T1R (aOR: 0.85, 95% CI: 0.17, 4.17), or T2R (aOR: 2.41, 95% CI: 0.29, 20.0). Vitamin D deficiency and vitamin A insufficiency were also not statistically associated with reactions. However, vitamin deficiencies and helminth infections were prevalent in these patients, suggesting a potential role for additional treatment interventions. Studying reactions prospectively may further clarify the role of comorbidities in the clinical presentation of leprosy.

INTRODUCTION

Brazil bears a large proportion of the global burden of leprosy, consistently reporting the second highest number of new cases after India. In 2016, Brazil had 25,218 new cases of 214,783 worldwide.[1] Leprosy, caused by infection with *Mycobacterium leprae*, produces a complex spectrum of disease manifestations and continues to cause severe disability and long-term morbidity in endemic areas. One of the most pressing questions is why some patients are more susceptible to type 1 and type 2 leprosy reactions than others. Because these severe immunologic complications are a significant cause of disability and irreversible damage, identifying risk factors for leprosy reactions is critically needed.

One-third to one-half of patients with leprosy will experience either type 1 reactions (T1R) or type 2 reactions (T2R).[2,3] Type 1 reactions (or reversal reactions) are characterized by an increase in cellular immunity and delayed hypersensitivity[4] and usually present with increased inflammation of skin lesions, neuritis, and nerve dysfunction.[5] Consequently, reversal reactions are a significant cause of nerve damage in patients with leprosy.[6,7] Type 2 reactions, also called erythema nodosum leprosum, occur only in lepromatous (LL) and borderline lepromatous (BL) cases. They are characterized by a systemic illness with immune complex formation, resulting in painful skin nodules, fevers, nerve inflammation, and other systemic symptoms.[6]

Comorbid conditions, such as minor bacterial infections, viral hepatitis, and HIV, have been implicated in the etiology of leprosy reactions, although results are mixed.[8–12] A recent cross-sectional study in Indonesia showed an association between soil-transmitted helminths (STH) and a higher occurrence of T2R.[13] This study also showed a higher odds of STH in multibacillary (MB) versus paucibacillary leprosy cases, an association which Diniz et al.[13] also demonstrated in Brazil. On the other hand, Hagge et al.[14] found a lower risk of reactions in STH-positive participants.

Although T1R and T2R can each have both Th1- and Th2-mediated cytokines,[15] the predominant response is cell-mediated (Th1) for T1R and immune complex formation for T2R. The immune response to chronic helminth infections is associated with an upregulation of Th2 cytokines and a suppression of the cell-mediated Th1 response.[16–18] Bystander effects on the immune response to another infection (in this case, leprosy) with the consequent shift to Th2 predominant response could then theoretically predispose a coinfected individual to a risk of reactions, especially T2R.[19] Likewise, micronutrient deficiencies can affect the immune response in the setting of various infectious diseases.[20–22] In the case of leprosy, vitamin A deficiency, which also suppresses cell-mediated immunity, could lead to a Th2 prominent response with a subsequent shift to the MB end of the spectrum, which has been shown from observational studies.[23,24] In addition,

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Mandal et al.[25] showed lower vitamin D3 levels and vitamin D receptor (VDR) expression in those with T2R.

Given the immune consequences of comorbidities such as helminth infections and micronutrient deficiencies, we conducted a pilot epidemiologic study to identify associations between them and either T1R or T2R in patients with MB leprosy infection. Other risk factors, such as age, gender, race, and socioeconomic status, were also investigated.

MATERIALS AND METHODS

Study site and population. This case–control study was conducted in Belo Horizonte, Brazil, at the Hospital Eduardo de Menezes, the regional public referral center for leprosy for the state of Minas Gerais (MG). The hospital is supported by funds from the Brazilian Ministry of Health and is one of the national reference centers within the Ministry's Leprosy Control Program. Minas Gerais is located inland in southeastern Brazil and is the second most populous state in the country with the fourth largest geographic area. Some regions in MG are endemic to both leprosy and *Schistosoma mansoni* infection as well as other helminths.[26–28] The clinic serves patients from the metropolitan area of Belo Horizonte as well as from all over the state. Patients typically present with complex symptoms that local physicians may not feel comfortable managing.

Eligible study participants included patients with MB disease, defined by the WHO as leprosy presenting with five or more skin lesions.[4] Cases were defined by the attending dermatologist as borderline tuberculoid (BT), borderline borderline (BB), BL, and LL,[29] consistent with the Ridley–Jopling classification system.[29] Borderline tuberculoid cases were only included if they were considered MB by the diagnosing clinician. Type 1 reaction was defined as having evidence of inflamed skin lesions or neuritis without systemic symptoms and T2R was defined as having evidence of painful skin nodules (erythema nodosum) and any of the following symptoms: fevers, arthralgias, or neuritis.[4] Those with mixed reactions had evidence of both reactions as defined by the clinician caring for the patient. Controls were defined as patients with MB disease who either never had a T1R or T2R, or were reaction free (and no reaction treatment) for 1 year or longer. Participants were recruited at any point in their leprosy treatment (newly diagnosed, on multidrug therapy (MDT), or completed MDT). Exclusion criteria for both cases and controls included pregnancy, age < 18 years, and paucibacillary disease. All patients on corticosteroid therapy were routinely given a regimen of ivermectin in case of *Strongyloides* infection per clinic protocol.

Data collection. Patients were recruited from July through December 2015 and asked to participate at a regularly scheduled clinic visit. Because patients had monthly clinic visits over the course of the study, most patients were eligible to participate. Written informed consent was obtained by Brazilian investigators. Height and weight were measured for each patient by trained investigators. Patients removed any jackets and shoes before weighing and if a participant was wearing jeans, this was documented, and 1 kg was subtracted. Body mass index (BMI) was then calculated for each patient. Questionnaires included basic demographic questions on race, occupation, socioeconomic status, place of residence (urban versus rural, district), and education. A detailed food consumption frequency questionnaire (FFQ) (validated for use in Brazil) was also administered to evaluate three food groups: 1) energy-supplying foods: carbohydrate and fats (cereals, sugars, roots, tubers, fats, and oils), 2) bodybuilding foods: proteins (milk, meat, poultry, eggs, fish, legumes, and groundnuts), and 3) repairing and maintenance foods: vitamins and minerals (vegetables and fruits). The results of the FFQ were evaluated according to the guidelines of the Food Guide for the Brazilian Population.[30] Details from the medical record included the type of leprosy, the presence of nerve damage, presence of reaction and which type (Type 1, Type 2, or mixed), date of leprosy diagnosis and reaction (where applicable), and current medications.

Blood samples were taken by venipuncture to test for anemia (hemoglobin), inflammation (C-reactive protein [CRP]), and micronutrient deficiencies (Vitamin A, vitamin D, and iron). In addition, serum samples were used to evaluate the exposure to *S. mansoni*, *Strongyloides stercoralis*, and/or *Ascaris lumbricoides* by IgG4 ELISA assay. The participants self-collected their stool samples at home on three consecutive days to identify any STH or *S. mansoni* infection.

Infection diagnosis. Infection of *S. mansoni* and STH was diagnosed by the Kato–Katz (K-K) and Hoffman–Pons–Janer (HPJ) methods of egg detection.[31] The stool samples were collected and transported in cups containing 10% formaldehyde vapor (Coproseco®) that preserves stool samples for up to 30 days at room temperature. Kato–Katz is a gold standard method and involves the analysis of two slides from one fecal sample. Although MG is an endemic region for schistosomiasis, prevalence is on the decline and infections may present with low intensity. Therefore, the standard K-K method can underestimate the prevalence. Because of that, we used a variant of this technique to increase the test sensitivity. Instead of one fecal sample, three samples were collected in one cup, which were mixed and homogenized in the laboratory. Six slides from this mixture were prepared using the K-K technique and examined. The remainder of this pooled sample was processed by Hoffman–Pons–Janer method, based on a spontaneous sedimentation.[32] The number of slides greatly increases the sensitivity of the method.[33] The HPJ method is used in numerous laboratories because it is easier to perform, has low cost, and is effective to diagnose protozoans and helminths infections, including *S. mansoni*, which have heavy eggs.[34] In our study, serological tests complemented the parasitological tests, as recommended by the Brazilian surveillance program in schistosomiasis in areas of low prevalence and lower intensity infections. Serologic evidence of previous *S. mansoni*, *A. lumbricoides*, and/or *S. stercoralis* infections was evaluated by ELISA assay, determining the IgG4 reactivity against *S. mansoni*–soluble adult worm antigens (SWAP), soluble antigens from tegument of *A. lumbricoides* adult worms (Al-teg), and soluble *Strongyloides venezuelensis* infective larvae antigens (L3-SV), respectively. Published data have shown that ELISA assay using IgG4 reactivity against soluble helminth antigens has been associated with chronic active infection.[35–39] Likewise, ELISA detecting IgG4 to soluble extract of *S. venezuelensis* infective larvae has been shown to accurately diagnose human strongyloidiasis.[39] Briefly, 96-well plates (Nunc-Maxisorb Nagle Nunc International, Rochester, NY) were sensitized overnight with 10 μg/mL of SWAP or Al-teg or L3-SV antigens in 0.1 M carbonate–bicarbonate buffer (pH 9.5) and blocked with

phosphate buffer (PBS) containing 1% bovine serum albumin (BSA, Sigma). Serum samples of the participants were diluted 1:100 in PBS containing 0.1% BSA and tested in duplicate. Bound antibody was detected by biotin-conjugated mouse antihuman IgG 4 (Sigma) followed by streptavidin–alkaline phosphatase. The threshold reactivity was determined by the absorbance average plus four SDs obtained from serum samples of six healthy volunteers who had been exhaustively tested for helminth infection by parasitological and/or molecular tests. We did not use a specific scotch tape test to recover *Enterobius* infections as these are not common in adults.

Nutritional testing and inflammation correction. Anemia is defined as a condition where the level of hemoglobin is < 12 g/dL for women and < 13 g/dL for men as per the WHO guidelines.[40] Concentrations of 25-hydroxyvitamin D, ferritin, and retinol were measured to identify deficiencies in vitamin D, iron, and vitamin A, respectively. C-reactive protein was measured to account for inflammation, given the effects of inflammation on retinol and ferritin, which are themselves acute phase proteins; ferritin concentrations increase with increasing CRP concentrations, whereas retinol concentrations decrease with increasing CRP concentrations.[41,42] A regression correction for inflammation was employed using a recently published approach that accounts for the effects of CRP on ferritin, as described elsewhere.[41,43] For ferritin and CRP, the Pearson correlation coefficient was 0.12 ($P = 0.30$); however, for retinol and CRP, the coefficient was -0.09 ($P = 0.43$). Although only weakly correlated, we still corrected the ferritin for CRP, given the high degree of inflammation present in the sample, but we did not correct in the cases of retinol because it was even weaker and it is generally not recommended to correct retinol for inflammation in adults.[44] Vitamin D deficiency was defined as 25-hydroxyvitamin D < 20 ng/dL. Vitamin A insufficiency was defined as a retinol level < 0.3 mg/dL and vitamin A deficiency as retinol < 0.2 mg/dL according to accepted guidelines.[41] Iron deficiency was defined as inflammation-adjusted ferritin < 15 ng/ mL.[41] An elevated CRP for this group was reported as greater than the lowest quintile of values for these participants (6 mg/mL).[41]

Statistical analysis. Few published data on leprosy-helminth coinfections limited the calculation of a sample size; however, an estimated helminth prevalence of 10% in controls and an odds ratio of six at a power of 80% and a confidence level of 95% would require a sample size of 66. All statistical analyses were performed in SAS version 9.4 (Cary, NC). Descriptive statistics and univariate analyses were performed with the main study variables and presence of reaction (either T1R or T2R) using chi-square, fisher's exact test, or *t*-test where appropriate. For the multivariate analyses, all reactions were first analyzed together and then T1R and T2R were separated to look for associations with helminth infection as the main exposure, and vitamin A insufficiency and vitamin D deficiency as secondary exposures. Iron deficiency was very rare, so was not included in the model. Other variables included age (continuous variable), gender, clinical type of leprosy (borderline versus LL), BMI (continuous variable), socioeconomic status based on monthly income (dichotomized for the logistic regression), race, presence of anemia, and smoking status. We did not have initial bacillary index (BI) at the time of diagnosis of leprosy and/or reaction for many patients; therefore, BI was not included in the model. For monthly income, two groups were considered in the logistic regression—those in the lowest category of income (< 1 × minimum wage) and those above this mark. For the T1R analyses, we compared cases with T1R to select controls. One model included controls which with either no reaction or those with T2R. A second model excluded T2R from the controls. Likewise, for T2R analyses, we compared cases with T2R to controls with either no reaction or T1R. In a second model, T1R cases were excluded. Because T1R and T2R are two different disease processes, we felt that it was appropriate to have models that included the other reaction as a control. Mixed infections were excluded for the individual T1R and T2R analyses. Model diagnostics included testing of collinearity and interaction, as well as an assessment of confounding using the change in estimates approach. Variables were sequentially dropped with this approach. A *P*-value of < 0.05 was determined to be significant. Adjusted odds ratios (aOR) and 95% CIs were calculated through multivariate logistic regression. Unadjusted OR for each variable in the final models were also calculated using chi-square or fisher's exact test where appropriate. All analyses were carried out using SAS v9.4 and OpenEpi v3.03a.

Ethics, consent, and permissions. Ethical approval was obtained from the Institutional Review Boards of Emory University and Faculdade da Saúde e Ecologia Humana. In addition, ethical approval was also granted by the Institutional Review Board at Hospital Eduardo de Menezes. Signed, informed consent was obtained for all study participants.

RESULTS

Participant characteristics. Over the 6-month study period, 53 patients with active leprosy reactions and 20 patients without reactions were recruited. Demographic and clinical variables of the study participants are described in Table 1. Among those with reaction, 24 (45.3%) had T1R, 21 (39.6%) had T2R, and eight (15.1%) had a mixed reaction of both types. Three of those without active reactions had had a reaction treated more than a year prior but had been asymptomatic off treatment for at least a year. The mean age among all study participants was 51.3 years (SD 14.4) and 53 (72.6%) were male. Most of the patients with reaction (68.4%) were first diagnosed after multidrug (MDT) treatment was completed (information available for 35 patients). Among the demographic and clinical study variables, only the clinical type of leprosy was statistically different among those with and without reactions on univariate analyses (Tables 1 and 2). The distribution of the clinical type of leprosy showed a higher percentage of LL disease in those with reaction and a higher percentage of BT disease in those without reactions (Table 1). Results from the stool and serum testing are outlined in Table 2. Of the 43 stool samples returned for analysis, only one was found to have a helminth infection and was identified as hookworm. We were unable to draw blood on one participant; therefore, 72 patients had blood drawn for micronutrients, helminth serology, complete blood counts, and CRPs. Serologic evidence of helminth infection was present in 11 of 72 (15.3%) (Table 2). Anemia was present in 21 participants (28.8%). About two-thirds of the patients ($n = 45$) were on corticosteroid therapy at the time of enrollment.

Micronutrient testing revealed a high burden of vitamin D deficiency ($n = 38$, 52.1%) and an 11.0% prevalence of

TABLE 1
Main demographic variables of patients with either type of active reaction and those without reaction

Variable	Reaction (n = 53)	No reaction (n = 20)	Total (n = 73)	P-value
Age (years), mean (SD)	50.2 (14.3)	54.1 (14.2)	51.2 (14.4)	0.33
Gender, *n* (%)				
Male	41 (77.4)	12 (60.0)	53 (72.6)	0.14
Type of reaction, *n* (%)				
Type 1	24 (45.3)	N/A	N/A	N/A
Type 2	21 (39.6)			
Mixed	8 (15.1)			
Clinical HD, *n* (%)				
Borderline tuberculoid (≥ 5 lesions)	5 (9.4)	5 (25.0)	10 (13.7)	0.02
Borderline borderline	13 (24.5)	9 (45.0)	22 (30.1)	
Borderline lepromatous	3 (5.7)	2 (10.0)	5 (6.9)	
Lepromatous	32 (60.4)	4 (20.0)	36 (49.3)	
BI*				
1st recorded: mean, SD	2.8 (1.8)	2.0 (1.4)	2.62 (1.7)	0.10
BI ≥ 4, *n* (%)	16 (32.7)	3 (18.8)	19 (29.3)	0.36
Race, *n* (%) (*n* = 72)				
African descent	20 (37.8)	9 (45.0)	29 (39.7)	0.57
White	15 (28.3)	3 (15.0)	18 (24.7)	
Mixed	16 (30.2)	8 (40.0)	24 (32.9)	
Refused	1 (1.9)	0	1 (1.4)	
Unknown	1 (1.9)	–	1 (1.4)	
Residence, *n* (%)				
Urban	40 (75.5)	15 (75.0)	55 (75.3)	0.97
Rural	13 (24.5)	5 (25.0)	18 (24.7)	
Monthly income,† *n* (%)				
< 1	13 (24.5)	7 (35.0)	20 (27.4)	0.65
1 to 3	35 (66.0)	11 (55.0)	46 (63.0)	
3 to 5	5 (9.4)	2 (10.0)	7 (9.6)	
> 5	0	0	0	
Body mass index, kg/m^2 mean (SD)	26.2 (4.0)	24.9 (4.4)	25.8 (4.1)	0.29
Smoking, *n* (%)				
Yes	16 (30.8)	4 (20.0)	20 (28)	0.56
Education, *n* (%)				
None	3 (5.8)	2 (10)	5 (6.9)	0.76
Primary	41 (76.9)	14 (70.0)	55 (75.3)	
Secondary	7 (13.5)	3 (15.0)	10 (13.7)	
Beyond	2 (3.9)	0	2 (2.7)	
Unknown	–	1 (5.0%)	1 (1.4)	
Disability grade, *n* (%)				
0	17 (32.1)	8 (40.0)	25 (34.3)	0.80
1	14 (26.4)	5 (25.0)	19 (26.0)	
2	22 (41.5)	7 (35.0)	29 (39.7)	
Active corticosteroid use,§ *n* (%)	36 (73.5)	9 (47.4)‡	45 (66.2)	0.04
Stage of treatment when diagnosed without reaction,‖ *n* (%)				
Within first 6 months	7 (20.0)	N/A	N/A	–
Second 6 months	4 (11.4)	–	–	–
After multidrug therapy completion	24 (68.6)	–	–	–

BI = Bacillary index. P-values describing differences were determined from t-test, chi-square, or Fisher's exact test where appropriate, and considered significant if < 0.05.
* Information missing for 28 participants.
† Definition: categories of monthly income determined by Brazilian minimum wage, with < 1 being below minimum wage and > 5 more than five times the minimum wage.
‡ Used for neuropathic symptoms in the absence of reactions.
§ Information missing for five participants.
‖ Information missing for 15 participants.

vitamin A insufficiency (n = 8). There were only three cases of vitamin A deficiency, all in patients with reactions. Only one patient had iron deficiency (Table 2). Inflammation was common with 92% of participants having an elevated CRP (Table 2). The results of the FFQ showed that protein- and carbohydrate-containing foods were the most commonly consumed, eaten by 100% of the patients. Fruit was consumed by 72.1% and vegetables by 80.3%.

Results of the multivariate analysis. *All reactions.* The first model grouped all reactions together and compared them with controls. Variables remaining in the model are shown in Table 3. Age, gender, anemia, smoking status, race, socioeconomic status, and BMI dropped out of all the models. Vitamin A insufficiency was retained in all models because of the hypothesized immune consequences of low vitamin A levels. Helminths (aOR: 1.36, 95% CI: 0.22, 8.33) and vitamin A (aOR: 2.88, 95% CI: 0.28, 29.84) were not found to be associated with reactions, but borderline leprosy was found to be associated with less reaction (aOR: 0.17, 95% CI: 0.05, 0.58).

Type 1 reaction. Variables that remained after model diagnostics are found in Table 4. The odds ratio of those with T1R having a helminth infection was not statistically significant at 0.85 (95% CI: 0.17, 4.17). Likewise, neither vitamin A insufficiency (aOR: 3.76, 95% CI: 0.53, 26.57) nor vitamin D deficiency (dropped from the model) was statistically

TABLE 2

Clinical laboratory results among those with reactions and those without reactions

Variable	Reaction	No reaction	Total	*P*-value
Hemoglobin (g/dL), mean (SD)	14.0 (2.0)	14.2 (2.0)	14.0 (2.0)	0.75
Presence of anemia, *n* (%)	17 (32.8)	4 (20.0)	21 (28.8)	0.31
Stool positive for helminth Infection, *n* (%) (*n* = 43)	1 (3.0) (hookworm)	0	1 (2.3)	0.77
IgG4 reactivity against helminth antigens				
Schistosoma (soluble adult worm antigen), *n* (%)	4 (7.7)	1 (5.0)	5 (6.9)	ND
Strongyloides, *n* (%)	2 (3.8)	1 (5.0)	3 (4.7)	ND
Ascaris, *n* (%)	5 (9.4)	0	5 (6.9)	ND
Any evidence of Helminth infection*, *n* (%)	9 (17.0)	2 (10.0)	11 (15.3)	0.44
Self-reported history of any parasitic infection, *n* (%)	13 (25.0)	2 (10.0)	15 (20.8)	0.21
Vitamin D deficiency, *n* (%)	27 (50.9)	11 (55.0)	38 (52.1)	0.48
Vitamin A insufficiency, *n* (%)	7 (13.2)	1 (5.0)	8 (11.0)	0.30
Vitamin A deficiency, *n* (%)	3 (5.7)	0	3 (4.1)	0.27
Inflammation-corrected Ferritin, ng/mL, mean (SD)	128.3	168.5	139.3 (109.1)	0.16
Iron deficiency, *n* (%)	1 (1.9)	0	1 (1.4)	0.54
CRP, mg/L, mean (SD)	26.4 (32.4)	14.1 (10.1)	23.1 (28.6)	0.10
CRP > 6 mg/L, *n* (%)	50 (94.3)	17 (85.0)	67 (91.7)	0.19

CRP = C-reactive protein. *P*-values describing differences were determined by *t*-test, chi-square, or Fisher's exact test where appropriate, and considered significant if < 0.05. Serum samples had a total sample size of 72 (52 with reaction and 20 without reaction) and stool samples had a total sample size of 43 (33 with reaction and 10 without reaction).

* Either stool positive for ova and parasite or serologic evidence.

associated with T1R. A history of borderline disease was significantly associated with T1R, with an aOR of 3.17 (95% CI: 1.06, 9.51). When the model was repeated using only those without reactions as controls, there were still no statistically significant associations with helminth infection or vitamin A insufficiency and T1R (Table 4).

Type 2 reactions. Helminth infection and T2R were not associated on multivariate analysis with an aOR of 2.41 (95% CI: 0.29, 20.0). Neither vitamin D deficiency (dropped from final model) nor vitamin A insufficiency (aOR: 0.35, 95% CI: 0.02, 6.32) was associated with T2R (Table 5). Again, the type of leprosy was found to be significantly associated with T2R, with borderline disease much less likely in those with T2R (aOR: 0.02, 95% CI: 0.002, 0.15). When the model was repeated excluding T1R from the controls, the results were very similar and also showed no statistically significant associations with helminth infection or vitamin A insufficiency and T2R (Table 5).

DISCUSSION

This pilot case–control study provides a picture of the demographic and clinical characteristics of 73 patients with and without reactions in a leprosy referral clinic in MG, Brazil. The burden of helminth coinfection, vitamin A insufficiency, and vitamin D deficiency in MB leprosy has rarely been described.

TABLE 3

Model 1: Multivariate logistic regression model with any reaction (type 1, 2, or mixed) as the outcome and helminth infection as the exposure, with crude and aOR

MODEL 1	Crude OR	95% CI	AOR	95% CI
Helminth*	1.88	0.37, 9.60	1.36	0.22, 8.33
Vit A insufficiency	2.89	0.33, 25.13	2.88	0.28, 29.84
Borderline disease	**0.16**	**0.05, 0.56**	**0.17**	**0.05, 0.58**

OR = odds ratio. Values in bold represent significant results with a *P*-value < 0.05.

* Defined as either a positive result on stool ova and parasite examination or serology for *Schistosoma*, *Strongyloides*, or *Ascaris* infections.

Vitamin D deficiency was common in our study, with more than 50% of participants affected even with using the lower limit of accepted values for 25(OH)D.[45,46] In prior studies, vitamin D deficiency has been associated with both MB disease and reactions, possibly because of the prominent role of VDR in the pathogenesis of leprosy.[25,47] Vitamin A insufficiency was more common than vitamin A deficiency and was present in 11% of study participants. Although there were no associations between reactions and these deficiencies in this pilot study, the prevalence of these vitamin deficiencies call for larger studies comparing patients with leprosy with healthy controls, as vitamin supplementation could be a straightforward adjunctive treatment for both leprosy and associated reactions.[20,24] It is also possible that *M. leprae* infection itself could be affecting the levels of these micronutrients, through increased losses in the urine, increased utilization, or impaired absorption.

We also found a 15% prevalence of active or recent helminth infection through measurements of IgG4 to *Strongyloides*, *Schistosoma*, *and Ascaris*, which is much higher than the most recently reported prevalence of helminths (< 2%) in urban areas of this state.[26] With more focus on the potential

TABLE 4

Model 2: Multivariate logistic regression model with T1R as the outcome and helminth infection as the exposure

MODEL 2	aOR†	95% CI	aOR‡	95% CI
Helminth*	0.85	0.17, 4.17	1.44	0.21, 9.78
Vitamin A insufficiency	3.76	0.53, 26.57	3.57	0.34, 37.44
Borderline disease	**3.46**	**1.06, 9.51**	0.57	0.14, 2.38

aOR = adjusted odds ratios; T1R = type 1 reaction; T2R = type 2 reactions. Values in bold represent significant results with a *P*-value < 0.05. Mixed reactions were not included. T2R are included as controls in the first column results and excluded as controls in the second column resultsBolded results represent significant results with a *P*-value < 0.05. Mixed reactions were not included.

* Defined as either a positive result on stool ova and parasite examination or serology for *Schistosoma*, *Strongyloides*, or *Ascaris* infections.

† Control group is those without T1R (includes those with no reaction or those with T2R).

‡ Control group is those without any reaction.

TABLE 5

Model 3: Multivariate logistic regression model with T2R as the outcome and helminth infection as the exposure

MODEL 3	aOR†	95% CI	aOR‡	95% CI
Helminth*	2.41	0.29, 20.0	3.16	0.14, 70.67
Vitamin A insufficiency	0.35	0.02, 6.32	1.24	0.003, 561.76
Borderline dx	**0.02**	**0.002, 0.15**	**0.01**	**0.001, 0.13**

aOR = adjusted odds ratios; T2R = type 2 reactions. Values in bold represent significant results with *P*-value < 0.05. Mixed reactions were not included. Type 1 reaction are included as controls in the first column results and excluded as controls in the second column results.

* Defined as either a positive result on stool ova and parasite examination or serology *for Schistosoma*, *Strongyloides*, or *Ascaris* infections.

† Control group is those without T2R.

‡ Control group is those without reaction.

role of various coinfections, such as HIV, viral hepatitides, and bacterial infections in leprosy reactions, this higher prevalence of helminth infection in this patient population requires further attention.[10-12] Although the underlying immune mechanisms of leprosy reactions are complex, T1R are generally associated with a robust cell-mediated immune response and T2R are associated with immune complex formation and more of a humoral immune response and may have both Th1 and Th2 cytokines.[5,23] As both vitamin A deficiency and helminth infections are associated with suppression of the Th1 immune response, theoretically, a higher risk of T2R and possibly lower risk of T1R could be hypothesized. This fits with the results of Oktaria et al.,[13] who found an increased risk of T2R in helminth-positive patients in Indonesia. Another study (Hagge et al.) investigated helminth coinfection and reactions in Nepal. Although they found an inverse relationship with leprosy reactions and STH coinfection, they grouped T1R and T2R together in their analyses, and overall, there was a much higher prevalence of T1R in their sample (only 13 of 69 reaction-positive patients had T2R).[15] Therefore, their results could be interpreted in the context of an overall prominent T1R, which would make sense given the suppression of a cell-mediated response with chronic helminths. It is also not clear whether prior mass treatment for STH (and hence being negative at the time of evaluation) may have influenced these results. Their conclusion that all leprosy reactions are associated with treatment of an STH is not necessarily supported by their data, especially with the lack of immunological assays to explain their hypothesis.[15] In addition, although Diniz et al.,[14] an older study in Brazil on STH-leprosy coinfections, did not analyze leprosy reactions, they found that STH-leprosy coinfected patients were more likely to have MB infection. Associated cytokine analyses showed a stronger Th2 response in these coinfections.[14] Therefore, although our study did not show the expected association between T2R and helminths, the shift to the Th2 immune response seen in the Diniz study deserves further investigation in both the context of leprosy reactions and the clinical presentation of leprosy.

Whereas Oktaria et al. used similar techniques to our study for helminth diagnosis (KK and formalin–ether), Hagge et al. used a different method to diagnose helminths (PCR-based), which may limit the comparability of these studies. Therefore, because the onset of reaction could have happened months to even years prior, serological tests have the advantage of measuring overall past exposure to infection, which may have set the groundwork for a shift in an immune response, even if active infection was not detected at the time of evaluation. Given the co-endemicity in many areas of the world as well as the strong immune response of schistosomiasis,[16,48] this is an important topic of future studies.

In addition, a striking finding was the amount of inflammation in the study patients, with 92% of participants having elevated CRP, especially given the fact that 44 (62%) participants were taking corticosteroids at the time of the study. Type 2 reaction has been associated with high CRP levels.[49] However, higher median levels of CRP have been seen in non-reactional states as well.[50] Longitudinal studies of CRP with additional inflammatory markers could help elucidate associations and possibly uncover ways to mitigate this strong inflammatory response. Consistent with prior studies, our analyses showed borderline disease (BT, BB, and BL) to be a risk factor for T1R and our T2R analyses demonstrated LL disease to be associated with a higher likelihood of T2R.[12] Other variables such as age and gender were not associated with either type of reaction in our study. Prior studies provided mixed results for these variables without a clear predilection according to gender or age-group.[51–54]

Limitations of this study include the small sample size and uneven distribution of overall cases of reactions (T1R and T2R) and controls. Because the dermatology clinic at the Hospital Eduardo de Menezes is a leprosy referral center for the state of MG and because reactions are often the most difficult complication to control, it makes sense that there would be a high number of cases with reactions referred to this clinic. Dividing the analysis between T1R and T2Rs gave a better distribution of cases and controls and made it possible to evaluate these individually; however, the low numbers still limit interpretation of findings. It is also possible that we underestimated the helminth infections in this population because only 43 (59%) patients returned their stool studies. Using serologic tools helped to mitigate this issue, although, and actually gave a better overall sense of a patient's exposure and infection history with helminths. In addition, the use of corticosteroids in some of the patients could have lowered the sensitivity of the antibody tests, although we did not find a significant difference between the prevalence of positive helminth serology between those taking prednisone or not (results are not shown).

Other important points to take away from this study include the fact that many of the patients in the case–control study were diagnosed with or still symptomatic with reactions after they had finished MDT, demonstrating the often-long period of time that patients can suffer from complications. Grade 1 or 2 nerve disability was found in most of these patients (Table 1), whether with reactions or not, again pointing to the long-term sequelae and the need for better diagnostics, case finding, and management of complications. This study also shows the complex nature of leprosy reactions and difficulties elucidating risk factors.

In summary, comorbid conditions of poverty, such as undernutrition and helminth coinfections, are prevalent in patients with leprosy and hypothesized underlying mechanisms of disease and immune responses may affect the predilection for leprosy reactions. Vitamin D deficiency could be a significant factor in the morbidity of leprosy, given the high prevalence found in our study and with the known role of VDR genes in the predilection for MB versus PB. Given the limitations of this and other observational studies, a randomized control trial with micronutrient supplementation or deworming medications at the time of diagnosis of leprosy would be an ideal way

to study this question further and determine if these interventions reduce (or even increase) the chance of T1R and T2R during a follow-up period. It is also important to pair epidemiologic and interventional studies with immune assays to better understand the underlying mechanisms of these complications. Identifying risk factors for leprosy reactions can reduce the burden of disability and improve the quality of life of those affected by leprosy.

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