REVISTA DO INSTITUTO DE MEDICINA TROPICAL DE SÃO PAULO

JOURNAL OF THE SÃO PAULO INSTITUTE OF TROPICAL MEDICINE

ORIGINAL ARTICLE

http://dx.doi.org/10.1590/S1678-9946201860067

High detection rate of new cases of multibacillary leprosy in Mato Grosso do Sul, Brazil: an observational study from 2001-2015

Leticia Ferrigolo Zanella[1], Iara Beatriz Andrade de Sousa[1], Marcelo dos Santos Barbosa[1], Odival Faccenda[2], Simone Simionatto[1], Silvana Beutinger Marchioro[1]

ABSTRACT

Leprosy remains a public health problem in Brazil, and the Mato Grosso do Sul State (MS) had the seventh highest rate of detection of new cases in the country in 2015 (26.59 per 100,000 inhabitants) which was classified as very high. This work aimed to determine the epidemiological characteristics of leprosy in MS. Descriptive statistics were performed with data from the Information System on Diseases of Compulsory Declaration (SINAN) between 2001 and 2015, with all patients included in the system serving as the sample. Clinical forms of multibacillary (MB) leprosy predominated in MS during the study period, with a clear positive trend from 2009 to 2015 and a peak in the detection rate of new cases (NCDR) in 2014 corresponding to 40.39 per 100,000 population ($p<0.001$). The most affected groups were men (56.7%) aged 20–59 years (70.52%), an economically active population. We observed that Northern MS had the highest overall NCDR in the State. In cities bordering other countries, NCDRs were significantly lower than in those of other analyzed cities. There was no dependency ratio correlating NCDRs in cities with higher or lower indexes with basic care coverage ($p=0.799$) and human development index ($p=0.887$). In conclusion, the large number of patients with MB leprosy indicates that the diagnosis of leprosy is delayed in MS, perhaps due to difficulties related to diagnostic methods. This situation contributes to the continuing prevalence of leprosy in MS.

KEYWORDS: Epidemiology. Leprosy. *Mycobacterium leprae.* Infectious diseases.

[1]Universidade Federal da Grande Dourados, Faculdade de Ciências da Saúde, Programa de Pós-Graduação Stricto Sensu em Ciências da Saúde, Dourados, Mato Grosso do Sul, Brazil

[2]Universidade Estadual do Mato Grosso do Sul, Programa de Pós-Graduação Stricto Sensu em Ensino em Saúde, Dourados, Mato Grosso do Sul, Brazil

Correspondence to: Leticia Ferrigolo Zanella
Universidade Federal da Grande Dourados, Faculdade de Ciências da Saúde, Programa de Pós-Graduação Stricto Sensu em Ciências da Saúde, Rua Ponta Porã, 1945, Jardim América, CEP 79824-130, Dourados, MS, Brazil
Tel: +55 67 3426-7349,
Mobile: +55 66 99971-4444

E-mail: leticiafzanella@gmail.com

Received: 4 June 2018

Accepted: 3 October 2018

INTRODUCTION

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, which predominantly affects the skin and peripheral nerves, giving rise to deformities such as muscle wasting and injuries in anaesthetized areas of the body[1]. Leprosy is a leading cause of preventable disabilities, leaving 3 million people disabled worldwide[2]. The bacillus has high infectivity and low pathogenicity, with a long incubation period ranging from 2 to 7 years. Transmission between humans is considered the main way to contract the disease, and people living in the same home as the bacillus carrier are the most susceptible[2,3]. Leprosy in children younger than 15 years old and the persistently high rate of disabilities in new cases are a robust indicator that leprosy transmission is continuing unabated[4,5].

For the purposes of diagnosis and definition of a therapeutic regimen with multidrug therapy (MTD), the World Health Organization (WHO) has established the classification of leprosy based on the number of skin lesions. Patients with up

to five lesions are grouped as having paucibacillary leprosy (BP) and those with more than five cutaneous lesions, are classified as having multibacillary leprosy (MB)[6,7]. Untreated patients with MB leprosy are likely the most important source of *M. leprae* transmission; it is estimated that people who have contact with these patients have a 5-10 times greater risk of acquiring the disease than the general population[8,9].

WHO's goal of eliminating leprosy by 1991 was reached globally, but the disease remains a public health problem in some countries[10]. The disease remains endemic in the Americas, with Brazil reporting the highest number of cases among all countries in the region. This high level of endemicity in Brazil is especially concentrated in the North, Northeast, and Central-West (CW) regions, with a very high detection rate of new cases (NCDR) and prevalence rate[11-13].

The CW region presented the second highest NCDR in the country in 2010, being classified as endemic with an unequal distribution[14]. In 2011, Mato Grosso do Sul State (MS) showed a reduction in the prevalence rate; however, despite this decrease, the State requires intensification of measures to eliminate the disease[15]. It is imperative to implement regional descriptive studies to better understand the distribution of leprosy at the local level, with a focus on aspects associated with the operational classification of the disease that may contribute to prevention, diagnosis and early treatment, increasing the effectiveness of control and avoiding disabilities and deformities caused by leprosy. Therefore, the aim of this observational study was to delineate the epidemiological profile and to determine the trend of leprosy in MS from 2001 to 2015, analyzed through the database of government notifications, correlating these data with social and health indicators.

METHODS

Study design

We conducted a retrospective cross-sectional ecological study of 11,516 patients with leprosy reported in MS between January 2001 and December 2015. Data were obtained from notifications to the Information System on Diseases of Compulsory Declaration (SINAN), Department of Informatics of the Brazilian Unified Health System (DATASUS), Instituto Brasileiro de Geografia e Estatistica (IBGE) and Department of Basic Care (DAB)[15,16].

Study area and population

The Mato Grosso do Sul State is located between the latitude -20°30'0 S and the longitude -55°0'0 W. It is organized in 79 cities with a population density of 6.86 inhabitants/km^2, an area of 357,145.534 km^2 and a population of 2,449,024 inhabitants[17]. The human development index (HDI) of MS is 0.729, which is considered average[18]. The unified health system (SUS) in MS is organized in 765 Family Health Strategy (FHS, primary care) teams distributed throughout the state. It is estimated that 76.6 % of the population has access to primary care[19].

Study variables

The variables of the study were: cases confirmed by year, city of diagnosis, operational classification, current therapeutic scheme, outcome, sex, age group, assessment of the degree of physical incapacity at diagnosis, HDI and FHS.

Data collection

Cases confirmed between January 2001 and December 2015 were retrieved from the SINAN online database, SinanWeb[16]. The use of this website was abandoned due to security problems, so that information became available through the DATASUS website[15]. We could confirm the cities belonging to MS State, the HDI and the number of inhabitants using data from IBGE[18]; data on FHS in the cities analyzed were retrieved from the DAB website[20].

Inclusion and exclusion criteria

As notification in Brazil is mandatory, we included all patients reported in SINAN with diagnosis of leprosy. Cases defined in SINAN as "diagnostic errors" were excluded from the analysis.

Statistical analysis

In this study, descriptive statistics were performed to analyze the obtained data. To describe the detection rate of new cases (NCDR) and the prevalence of leprosy, 95% confidence intervals were determined for binomial distributions[21]. For the prevalence rate, cases in a possible course of treatment were considered, including all the cases diagnosed in the year and two years prior to the year of evaluation, by 10,000 inhabitants from a certain geographic space. The NCDR of leprosy was calculated based on the number of cases detected in a year multiplied by 100,000 and divided by the total population in that year[22]. The chi-square test was used to verify if there was a significant difference in NCDRs between years, with 2001 as the base year, and

between the NCDRs of PB leprosy and MB leprosy in each year. The significance level was set at 5%[23]. A multiple linear regression was used to test the independence between response variables and control ones. A bilateral Mann–Whitney *U* test of independent samples was used to test the hypothesis[21]. The software used in the analyses was R-project for statistical computing (version 3.2.4).

Ethical considerations

Considering that this manuscript used only a database with aggregated information and no possibility of individual identification, the resolution 510/16 from the National Research Ethical Board - CEP/CONEP system[24] was fulfilled.

RESULTS

Epidemiological characterization

In this study, we found that most of the population diagnosed with leprosy was composed of males (56.7%; N=6,530). Of these, 61.84% (N=4,610) were affected by the multibacillary form (MB); most individuals with the paucibacillary form (PB) were females (52.85%; N=2,132). There was a predominance of patients between 20 and 59 years old (70.52%; N= 8,101), with a higher proportion of MB leprosy among patients over 60 years old (25.45% MB vs. 16.71% PB). The majority of patients who completed treatment were considered cured according to SINAN's criteria (85.91%; N= 7,338); patients with the PB form had a greater chance of cure than those with the MB form (p<0.001). This finding may be related to the shorter treatment time in PB patients, increasing the percentage of patients who completed treatment when compared to MB patients (Table 1).

Leprosy in Mato Grosso do Sul, the Central-West Region, and throughout Brazil

In the period from 2001 to 2015, 664,500 leprosy patients were notified nationwide (some from periods before the establishment of SINAN). Of these, 59.3% (N= 393,990) had MB leprosy, 40.36% (N= 268,237) had PB leprosy and 0.34% (N= 2,273) had an unknown situation (not notified or improperly notified). In the entire CW Region, 112,193 cases were confirmed, representing 16.89% of the cases in the country; cases in MS accounted for 10.14% of cases in the region. In the 15-year period, MS reported 11,374 patients (144 cases prior to SINAN), with a predominance of MB leprosy (65.3%; N= 7.424).

Prevalence, NCDR and physical disabilities due to leprosy in Mato Grosso do Sul

Highest prevalence rates were observed during the period from 2001 to 2004, when compared to later years of the study. A sharply decrease was observed from 2004

Table 1 - Epidemiological characterization of leprosy cases reported to the Information System on Diseases of Compulsory Declaration (SINAN) in the Mato Grosso do Sul State, Brazil between 2001 and 2015.

Variables		Total	%	PB		MB		p-value*
				N	%	N	%	
Sex	Male	6530	56.70	1902	47.15	4610	61.84	≤0,001
	Female	4986	43.30	2132	52.85	2845	38.16	
Age (years)	0 to 19	816	7.10	511	12.67	305	4.09	≤0,001
	20 to 59	8101	70.52	2849	70.62	5252	70.46	
	60 or older	2571	22.38	674	16.71	1897	25.45	
Outcome	Cure	7338	85.91	3217	90.85	4110	82.43	≤0,001
	Transf. to the same city	77	0.90	16	0.45	61	1.22	
	Transf. to another city	236	2.76	41	1.16	195	3.91	
	Transf. to another state	94	1.10	17	0.48	77	1.54	
	Transf. to another country	19	0.22	5	0.14	14	0.28	
	Died	162	1.90	13	0.37	147	2.95	
	Abandoned	350	4.10	138	3.90	212	4.25	
	Unspecified Transf.	265	3.10	94	2.65	170	3.41	

Abbreviations: Transf: transference; PB: paucibacillary; MB: multibacillary. *Association between the variables characterizing the sample and the type of leprosy (pauci and multi) were analyzed by the Chi-square test.

to 2005 and from 2009 to 2010, with a reduction of 84.5% and 93% respectively. From 2005 to 2007, the leprosy prevalence increased by 76.87% and decreased by 15.75% from 2007 to 2008. Between 2010 and 2012, the prevalence remained on the average of 0.14 per 10,000 inhabitants, however, during the period of 2012 to 2015, there was a sharply increase of this rate, going from 0.12 to 2.28 respectively, which represented an increase of 1,900 % in the period. This made the prevalence rate again similar to the average found between the years 2005 to 2009, which was 2.1 per 100,000 inhabitants (Table 2).

Regarding the NCDR, 2014 presented the highest value in the analyzed period, with an increase of 56%. When we observed the new cases detected in 2014 compared to 2007 (the highest and the lowest rate in the studied period, respectively), there was an increase of 70%, which means almost two times more new cases detected in 2014 in comparison to 2007 (Table 2). In the mildest form, PB leprosy, there was a considerable reduction of new cases from 2010 onward (p<0.001), with a decline of 68% during all the evaluated period (from 12.5 to 3.93). On the other hand, the NCDR from the MB form showed an increase of 59% (from 14.26 to 22.66) from 2001 to 2015. There was also a significant increase in the proportion of NCDR from MB cases in relation to the year 2001, from 2010 onward (p=0.001) (Table 2).

When confirmed cases were analyzed according to the current operational classification by the year of notification, we observed that the number of newly infected patients grew each year. However, in 2015 there was a significant decline compared to 2014 (p=0.044). PB cases showed a significant decline, while MB cases rose until 2014 (Figure 1).

In the period between 2001 to 2015, the average of NCDR in leprosy cases with grade 2 physical disabilities assessed at the time of diagnosis was 1.94 per 100,000

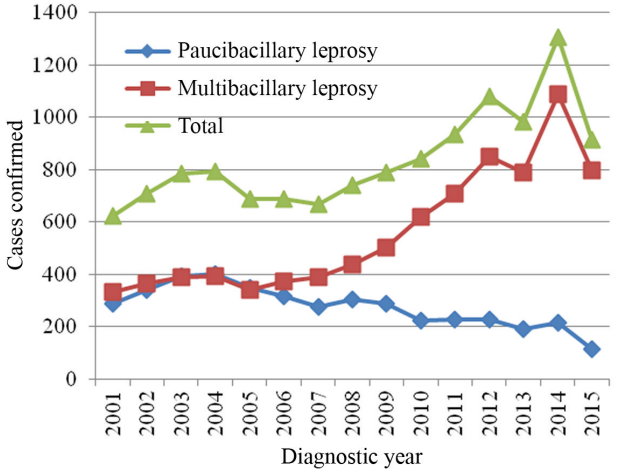

Figure 1 - Cases confirmed by the operational classification of leprosy in the Mato Grosso do Sul State, Brazil, from 2001 to 2015.

Table 2 - Prevalence, new case detection rate (NCDR) and degree 2 of leprosy disabilities o in the Mato Grosso do Sul State from 2001 to 2015.

Year	Prevalence[1]	NCDR[2]	p-value[3]	PB NCDR[2]	p-value[3]	MB NCDR[2]	p-value[3]	NCDR with Grade 2 of disability	p-value[3]
2001	27	26.76	-	12.50	-	14.26	-	1.52	-
2002	22.13	28.87	0.212	14.46	0.094	14.41	0.973	1.45	0.9674
2003	22.11	32.82	<0.001	17.00	<0.001	15.82	0.218	2.12	0.7510
2004	9.49	31.56	0.003	16.36	0.001	15.20	0.399	1.59	0.9681
2005	1.47	26.94	0.821	14.36	0.082	12.58	0.142	1.41	0.9484
2006	1.62	26.63	0.976	12.90	0.667	13.73	0.742	1.39	0.9388
2007	2.60	24.98	0.136	10.78	0.061	14.20	0.782	2.32	0.6802
2008	2.19	26.75	0.808	12.05	0.839	14.70	0.577	1.76	0.8937
2009	2.60	24.78	0.620	9.74	0.114	15.04	0.039	1.4	0.9436
2010	0.16	26.66	0.840	8.15	<0.001	18.50	<0.001	2.74	0.5502
2011	0.15	29.30	0.086	8.21	<0.001	21.09	<0.001	1.94	0.8198
2012	0.12	34.25	<0.001	8.66	<0.001	25.59	<0.001	1.76	0.8937
2013	3.0	26.05	<0.001	5.85	<0.001	20.2	<0.001	2.51	0.6183
2014	3.91	40.39	<0.001	7.39	<0.001	33.00	<0.001	2.48	0.6278
2015	2.28	26.59	0.220	3.93	<0.001	22.66	<0.001	2.75	0.5474

[1]Per 10,000 inhabitants. [2]NCDR: New case detection rate per 100,000 inhabitants, PB (paucibacillary), MB (multibacillary) leprosy. [3]Chi-square test comparing years.

population. However, Table 2 shows that there was a steady trend for this rate with little non-significant variation between the analyzed years (p>0.05) and the highest detection rate in 2015, presenting a value of 2,75 per 100,000 inhabitants, an increase of 80% in relation to the year 2001, classified as high and indicating a delayed early diagnosis of the disease.

Spatial analysis of leprosy in Mato Grosso do Sul

When analyzing the spatial distribution of leprosy, in addition to Pedro Gomes municipality with a rate of 200.05 per 100,000 inhabitants, two other cities presented an NCDR greater than 100 (Paranaiba and Navirai) and 5 had an NCDR above 50 (Coxim, Bodoquena, Bandeirantes, Agua Clara and Bonito), as shown in Table 3 and Figure 2A.

The cities of Pedro Gomes and Navirai had the highest NCDRs of PB leprosy, whereas one of the lowest rates in the State was found in Rio Negro and Campo Grande, with NCDRs of 5.07 and 6.00 per 100,000 inhabitants, respectively (Table 3). Using maps of the leprosy incidence in MS, we compared the spatial distribution according to operational classification (Figure 2B). We noted that there was a greater concentration of high MB leprosy incidence throughout the State compared to those of PB leprosy (Figure 2B).

We evaluated the number of confirmed leprosy cases in MS cities directly bordering another country and those with an indirect border (cities within a 150-km distance from a border but with no contiguous border areas) between 2001 and 2015, as well as the respective NCDRs. We found no significant differences among border cities (bordering Bolivia, Paraguay, and those with an indirect border) with respect to general NCDR of leprosy among non-border cities. However, in cities bordering Bolivia, PB leprosy NCDRs (13.58 per 100,000 inhabitants) were significantly higher than in cities with other borders (9.45 per 100,000 inhabitants); the NCDR for MB leprosy was lower (15.17 and 18.77 per 100,000 inhabitants, respectively). Cities with indirect borders had the lowest PB leprosy NCDRs (8.85 per 100,000 inhabitants) compared to other cities. When comparing border cities in MS with cities having the highest leprosy NCDRs in the state between 2001 and 2015, we observed that the NCDRs in border cities (28.55 per 100,000 inhabitants) were significantly lower than those of the other cities analyzed (88.82 per 100,000 inhabitants) (Table 3).

The comparison of cities with the highest leprosy NCDRs with those having the lowest incidences, and considering the HDI and FHS coverage, we observed that there was no significant dependency relationship of the variable NCDR response with the control variables FHS (p=0.799) and HDI (p=0.887). Nevertheless, Navirai, one of the cities with a NCDR greater than 100, had one of the lowest FHS coverage in that period.

Table 3 - Cities with the highest new case detection rate of leprosy, family health strategy (FHS) coverage and human development index (HDI) in Mato Grosso do Sul State, between 2001 and 2015.

City	New case detection rate[1]			FHS Coverage	HDI
	Total	PB	MB		
Pedro Gomes	200.05	36.19	163.86	77.49	0.67
Paranaiba	137.11	25.43	111.68	83.65	0.72
Navirai	118.22	59.84	58.25	46.58	0.70
Coxim	95.69	12.44	82.65	72.01	0.70
Bodoquena	86.67	26.87	59.80	70.01	0.67
Bandeirantes	75.06	9.26	65.80	87.74	0.68
Agua Clara	52.67	17.24	35.43	76.81	0.67
Bonito	52.52	16.64	35.88	53.64	0.67
Rio Negro	46.69	5.07	41.61	95.79	0.71
Alcinopolis	43.13	6.16	36.97	83.79	0.71
Novo Horizonte do Sul	40.9	8.67	32.23	84.38	0.65
Rio Verde de Mato Grosso	37.43	10.62	26.81	98.82	0.67
Campo Grande[2]	15.68	6.00	9.62	25.3	0.78
Dourados[3]	20.3	10.08	10.11	56.31	0.75

[1]Per 100.000 inhabitants. [2]Capital of Mato Grosso do Sul State. [3]Second largest city in the state. Abbreviations: PB: paucibacillary leprosy; MB: multibacillary leprosy; FHS: family health strategy coverage; HDI: human development index.

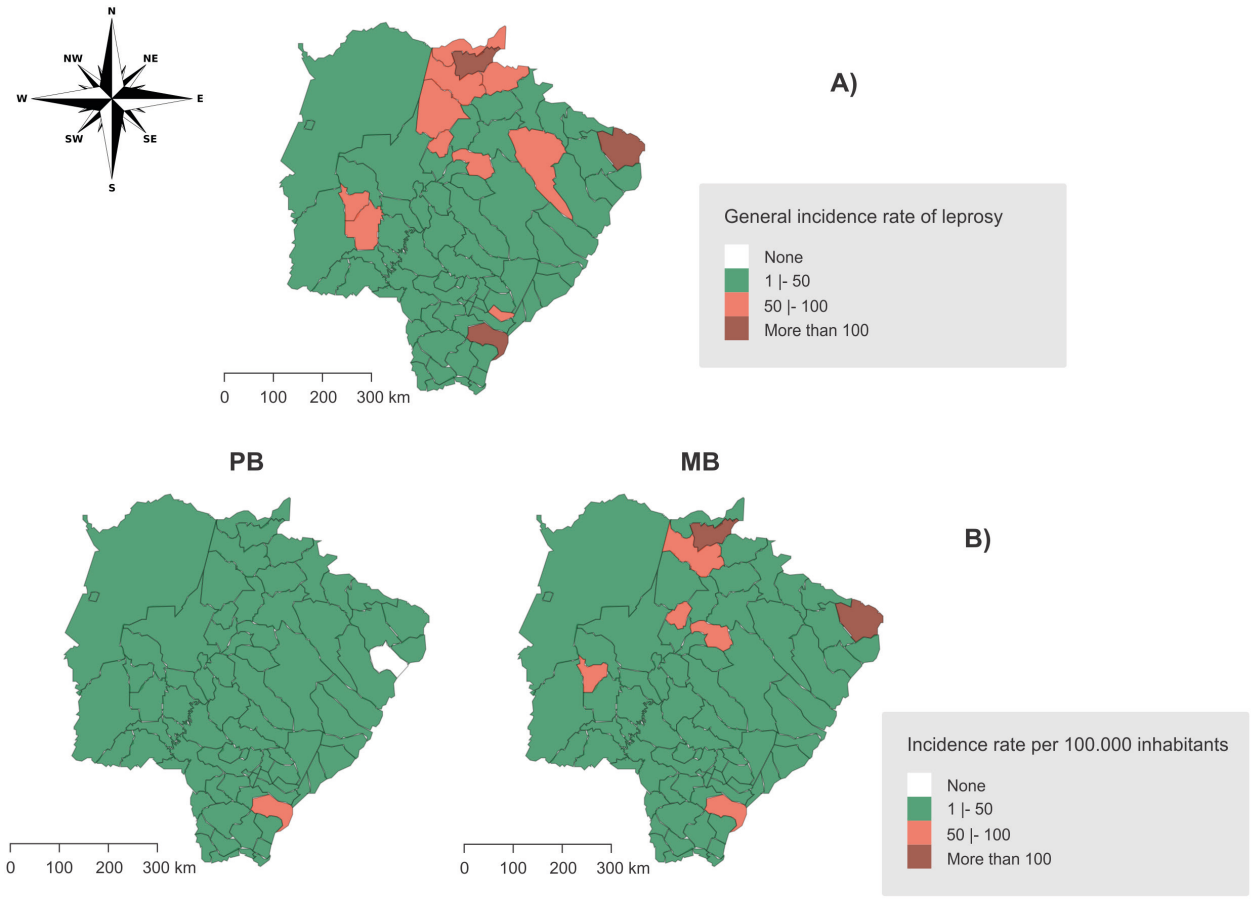

Figure 2 - Spatial distribution of the incidence rate of leprosy in Mato Grosso do Sul State, Brazil, between 2001 and 2015. A) General incidence of leprosy. B) Paucibacillary and multibacillary leprosy incidence. PB: paucibacillary leprosy; MB: multibacillary leprosy.

DISCUSSION

Brazil is the only Latin American country that has not achieved the goals proposed by the WHO for elimination of leprosy, although several control programs were created, and data presented by the Brazilian Ministry of Health have shown that the incidence in Brazil is slowly decreasing[25,26]. As far as we know, there are no temporal studies of epidemiological indicators covering long periods indicating trends for leprosy in Mato Grosso do Sul State. According to our results, the number of leprosy cases reported in MS between 2001 and 2015 was consistent with the national representativeness of the state's population (1.71% of cases in Brazil/ 1.28% of the national population)[18,27].

Young adults were the most affected by leprosy in this study, suggesting a negative impact on the economy as the disease can cause disabilities and symptoms that prevent people from working[28]. In 2015, Silva *et al.*[29], stated that the high number of patients with MB leprosy is alarming as it affects an economically active age group with greater likelihood of disease transmission[29]. According to a 1988 WHO report and recent study by Ramos *et al.*[30], although leprosy affects both sexes, men are affected more often than women with a ratio of 2:1[30]. These data were confirmed in our study population in which significantly higher rates of the MB form were found in men between ages 20 and 59 years (Table 1).

The prevalence rate of leprosy in MS decreased from 2001 to 2015, with emphasis in the period from 2004-2005, with a decrease of more than 80% (Table 2). In Brazil, in the same study period, there was a decrease of 20,000 new cases observed between 2003 and 2013 (40% reduction), coinciding with a period of significant decentralization of leprosy control activities in the country. From 2000 to 2011, a 284% expansion in the number of health centers that registered patients under treatment (from 3,327 to 9,445)[31] was reported. Although MS followed the trend of the country, with a reduction of prevalence, in 2015, the last year of evaluation in this study, MS had a medium prevalence of 2.28 cases per 100,000 inhabitants, the 5th highest rate in the country, and a very high NCDR, 26.59 cases per 100,000, inhabitants, representing the country's seventh highest NCDR[27].

The very high leprosy NCDR in MS, as well as in other States, indicates the continuing transmission of the disease in Brazil. Clinical MB leprosy predominates in MS, with a clear positive trend from 2009 to 2015, while PB cases are decreasing. The increase of MB leprosy is showing that prevention of transmission has not been achieved despite implementation of multidrug therapy (MDT) programs and the creation of several control programs[32]. The number of disabilities at diagnosis has also been highlighted in the study. During the evaluated years, an increase in the new diagnosed cases with degree 2 of incapacities was observed, reflecting a delay in diagnosis and treatment, what is consistent with the ongoing transmission of infection[33]. The reduction of new PB form cases and the increment of new MB cases, together with the permanence of patients diagnosed with grade 2 of incapacities, may also show that more people are been diagnosed at an advanced stage, suggesting difficulties related to diagnostic methods, lack of access to more complex or precise methods and deficient basic care networks. Consequently, numerous cases remain undiagnosed and untreated, and these individuals will act as continuing reservoirs of infection and have a profound impact on the maintenance of the chain of transmission[34].

It is worth mentioning that the Mato Grosso do Sul State has land borders (1,578 km) with two countries, Paraguay and Bolivia, and five other Brazilian states (Mato Grosso, Goias, Minas Gerais, Sao Paulo, and Parana). There is constant migration between countries and states from MS. For this reason, patients are diagnosed and treated for leprosy in MS regardless of their nationality, place of birth or legal status, and they are included in the state statistics. This situation could influence local NCDRs, which may be considered a limitation of the study, when the place of diagnosis is considered instead of the place of residence. However, we found no significant differences among border areas with Bolivia, Paraguay, as well as among indirect border areas in relation to the general NCDR, when among non-border cities were analyzed. In our study, only 11.6% of leprosy cases in MS occurred in cities bordering Paraguay. It is important to note that Paraguay has reached the WHO target for leprosy elimination, confirming that bordering this country is not a risk factor for leprosy[35].

In relation to the distribution of leprosy by city, cities in Northern MS have quite high NCDRs compared to other parts of the state. The cities diverge reasonably, showing a heterogeneous distribution; however, ratio determination was difficult as it was not possible to confirm endemicity or the use of different detection strategies. Spatial analysis of leprosy is of paramount importance to verify the endemic areas scattered throughout the state[12]. However, according to Ramos *et al.*[30], the spatial distribution of leprosy should be carefully analyzed owing to possible errors in detection, underreporting, and/or late diagnosis that may result in erroneous designation of low NCDR areas[36].

It is known that leprosy is directly associated with precarious health conditions and low socioeconomic status[34]. The knowledge about risk areas for leprosy reveals that the distribution is intimately linked to a number of factors that coincide for the maintenance of high rates of transmission and NCDR, including environmental, individual, socioeconomic and health service organization factors[30]. The results of this study showed that there were no significant differences in HDI and access to primary health care (HFS) between cities with higher and lower NCDR. However, three cities with more than 100 cases per 100,000 inhabitants were detected in the evaluated period: Pedro Gomes, Paranaiba and Navirai. All three cities are located in different parts of the state, bordering other states, on the North, South and East, been classified as hyperendemic regions. Not surprisingly, one of these cities, Navirai, has one of the lowest HFS coverage in that period. Although no differences were detected in the linear regression, these data may indicate that low coverage by family health strategies may have contributed to the high incidence of leprosy in this city.

Considering the findings of this study, it was possible to have a panoramic view of leprosy in MS State and identify possible risk areas. Despite the reduction in the leprosy prevalence rate, the NCDR with grade 2 disabilities and the multibacillary form NCDR had a considerably increase during the study period, highlighting the three municipalities, from different regions of the State, classified as hyperendemic for the disease. These findings suggest delays in case diagnosis and shortcomings in preventing disabilities, suggesting possible operational difficulties in controlling the disease. It is important to keep in mind that we used secondary data produced in different settings nationwide, thus leprosy classification methodology may vary according to the center diagnostic capacity. In addition, according to previous work that dealt with the national leprosy database, SINAN, there is a tendency to underestimate the real number of cases[13,25]. Although this may be considered a limitation, it is evident that a continuous active surveillance in this area is necessary, including strategies for strengthening the local leprosy control program and rebuilding professional expertise to achieve a successful reduction of the current leprosy detection rate in this region. This will prevent grade 2 disability cases, especially in hyperendemic municipalities, finally reducing the disease burden and preventing disabilities. Considering the early diagnosis and the

empowerment of this population, awareness about leprosy needs to be increased and people should be encouraged to work in partnership with the health services to deal with the problem. Furthermore, social institutions within these areas, such as churches, kindergartens and schools should be encouraged to work jointly with health services, as they are located in risk areas.

CONFLICT OF INTERESTS

The authors have no conflict of interests to declare.

FINANCIAL SUPPORT

Support Foundation for the Development of Education, Science and Technology in the Mato Grosso do Sul State (FUNDECT grant 092/2015).

AUTHORS' CONTRIBUTIONS

Leticia Ferrigolo Zanella: master responsible for the project, participated in all planning, execution and preparation of the manuscript; Iara Beatriz Andrade de Sousa: participated in the scientific construction of the manuscript, considering the steps of statistical analysis and revision; Marcelo dos Santos Barbosa: participated in the scientific construction and revision of the manuscript; Odival Faccenda: participated in the scientific construction of the manuscript, considering the steps of statistical analysis; Simone Simionatto: participated in the scientific construction of the project and revision of the manuscript; Silvana Beutinger Marchioro: advisor and supervisor of the project.

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