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Research for Acid-fast Bacilli as a Diagnostic Aid of Leprosy at the Cristalina, Goiás, Brazil

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Abstract

Introduction: Leprosy is a public health problem with Brazil ranking the second in total number of cases, only behind India. Leprosy is a disease not eradicated in Brazil. This study is aimed to analyze the data from bacilloscopy as a tool to aid in the diagnosis of leprosy in Cristalina-Goiás, Brazil.

Methods:A total of 266 new suspect cases of leprosy were analysedthrough bacilloscopy between 2005 to 2008. Bacilloscopy of skin scrapings was done in suspected cases after the Ziehl-Neelsen coloring. The bacterial index and Ridley-Jopling scale was performed.

Results and discussion: The disease is not homogeneous over the territory of Brazil, with regions showing tendencies in control. The detection rates of new cases remained elevated in the North, Midwest, and Northeast. The detection frequency by bacilloscopy in Cristalina was low (14.66%) for multibacillar cases.

Conclusions: Bacilloscopy was important in the diagnosis and indirectly in the clinical classification of different cases, however, the county health department should implement new methods of diagnosis in Cristalina, Goiás, Brazil.

Keywords: Leprosy, Detection, Brazil, *Mycobacterium leprae*.

Introduction

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, which affects mainly the skin and peripheral nerves, with high prevalence worldwide. *M. leprae,* is a slow growing intracellular bacillus, that causes a few infiltrative lesions with loss in sensitivity up to significant and disabling sequels. Leprosy burden declines in all endemic countries, while in Brazil the annual number of new cases is about the same in the last 5 years [1]. In 2008 in Mexico, a new species of mycobacteria that causes leprosy was reported, known as *M. lepromatosis* [2].

At the beginning of the decade of the 1990s, the World Health Organization (WHO) established the goal of reducing the prevalence of leprosy to less than one case per 10,000 individuals by the year 2000. Nowadays, leprosy is a health problem in only 15 countries (prevalence rate > 1 per 10,000), including Brazil [1].*M. leprae* transmits through the droplets (upper respiratory tract) or prolonged contact by lesion on skin or mucous membranes of the infected individuals into healthy individuals [2].

Depending on the immunity against leprosy, the disease may present within a spectrum of clinical manifestations that vary from a localized form [tuberculoid–tuberculoid (TT)] to a disseminated form [lepromatous leprosy (LL)] or one of three intermediate forms (borderline–tuberculoid, BT; borderline–borderline, BB; borderline–lepromatous, BL)[3]. Paucibacillary (PB) cases have a small number of bacilli in the body and are regarded as resistant to the infection compared to multibacillary (MB) cases, which has a high rate of bacillus growth. The clinical diagnosis of leprosy is based on morphological and topographic criteria of the lesions, associated with changes in skin sensitivity and thickening and/or pain of peripheral nerves, however, the gold standard for diagnosis is the bacilloscopy (microbiological confirmation) that is given by the presence of acid-resistant bacilli in the lymph smear [4].

For treatment purposes of leprosy, patients with more than five skin lesions are MB and those with up to five lesions are PB. This classification was suggested in places lacking laboratory physical structure, equipment, supplies and technical personnel trained to carry out an appropriate bacteriological examination [4]. The bacilloscopy for leprosy is considered as a complementary test for the identification of PB and MB cases of difficult clinical classification. Thus, the acid-fast bacilli (AFB) smear-positive case is defined as MB, however, the diagnosis is not excluded with the negative result on the baciloscopy [4].

Early diagnosis is helpful not only for the reduction in transmission but also in the prevention of physical disabilities [5]. The criteria for clinical diagnosis are associated with positive bacilloscopy that suggests treatment with multidrug therapy [6]. The bacteriological examination, with the search for AFB is the most useful complementary test in the diagnosis, where the result is important for diagnosis an aiding in the classification of the patient in the clinical spectrum[7]. This study aimed to

analyze the data from bacilloscopy as a tool to aid the diagnosis of leprosy in Cristalina-Goiás, Brazil.

Methods

From June 2005 to June 2008 were examined 266 slides (18, 0-10 years old, 26, 10-20 years old, 36, 20-30 years of age, 67, 30-40 years of age and 119 over 40 years of age) through the bacilloscopy technique from the Municipal Hospital Chaud Salles in Cristalina-GO, Brazil. This study was conducted with suspected leprosy patients who had lesions characteristic of the disease and were referred for dermatological examination. At the dermatology clinic, the clinical inspection consisted of a complete examination of the skin, nerve palpation, temperature and pain sensitivity test of extremities and any suspicious lesion. The collection of lymph through the cutting of skin scrapings was carried in the right ear (RE) and left (LE), right elbow (DC) and left(EC) and also in suspicious lesions (L). Craping samples for bacilloscopy were always collected by experienced professionals.

After collection, the material was stained by Ziehl-Neelsen and observed by optical microscope. The sample analysis technique consisted of five steps: collection, fixation, Ziehl-Neelsen staining, microscopy, reading, and interpretation. It was determined the bacterial index (BI) of each one by Ridley logarithmic scale, which ranges from 0 to 6 + (1+ at least 1 bacillus in every 100 fields, 2+ at least 1 bacillus in every 10 fields, 3+ At least 1 bacillus in every field,4+ At least 10 bacilli in every field, 5+ At least 100 bacilli in every field, 6+ At least 1000 bacilli in every field)[5].

Results

Of the 266 slides, 135 were from males (50.75%) and 131 (49.25%) females, where after examining 39 were positive [29 (74.35%) males and 10 (25.65%) female].Table 1 shows the prevalence of positive smears by age, when there was a male predominance and higher detection after 40 years of age. Table 2 shows the distribution of analysis according to the BI, noting the predominance of paucibacillary forms (85,33%). The majority of cases were in rural areas (25 cases, 64.10%) rather than in urban areas (14 cases, 35.90%) of the city.

Discussion

Leprosy in Brazil and in the state of Goiás, where reported by this study iscaused by *M. leprae*, however in southern Brazil there are reports of few cases of leprosy caused by *M. lepromatosis*[8]. The paucibacillary form was predominant; the high proportion of paucibacillary cases may reflect an improvement in clinical detection of new cases in a high endemic area [9]. The frequency rate by bacilloscopy was low (14.66%) for multibacillary cases. The health authorities decided the non obligation of microbiological confirmation by microscopy, which favors the early treatment of leprosy. Furthermore, the laboratory confirmation of leprosy was possible in only 30% of cases in Recife, 28% in Manaus, 41% in Maranhão but less than that found in Rio de Janeiro (78%) and in Uberaba (60%)[9-14].The detection rates of new cases remained elevated in the North, Midwest, and Northeast [4]. Since the introduction of multidrug therapy, endemic countries have been working with the possibility of eliminating the disease, however this is not what occurs in the majorities of municipalities in Brazil, including Cristalina. Currently, India, Brazil, Indonesia, Bangladesh, and Ethiopia are the top five countries that are home to more than 80% of the new leprosy cases [15].

It was noted the predominance of cases detected in the rural areas, followed by urban area. Silva [14] described that the distribution of cases showed 42 of the leprosy patients, 29 (69%) lived in town and 13 (31%) in villages (rural area). In this study we obtained a prevalence of detection in men. In Brazil, males are more affected by leprosy [16,17]. The endemicity of a region can be raised by observing the age of detection of cases where the onset of the disease in people younger than 14 years indicate a high

Table 1: Distribution of leprosy by age

Age	2005	2006	2007	2008	Total
0 to 10	0	0	0	0	0
10 to 20	1 (man)	1 (woman)	0	1 (men)	3 (7.69%)
20 to 30	2 (men)	2 (men)	0	0	4 (10.25%)
30 to 40	2 (1 men, 1 women)	3 (men)	1 (men)	1 (women)	7 (17.94%)
>40	6 (3 men 3 women)	7 (5 men, 2 woman)	4 (3 men, 1 woman)	8 (7 men, 1 woman)	25 (64.10%)
Total	11 7 men (63,63%), 4 women (36.36%)	13 10 men (76.92%), 3 (23.07%)	5 4 men (80%), 1 woman (20%)	10 8 men (80%), 3 women (20%)	39 29 men (74.35%), 10 women (25.65%)

Table 2: Distribution of leprosy analyzed by bacillary index(BI)

Bacillary Index	2005	2006	2007	2008	Total
0	58	62	47	60	227 (85.33%)
1+	0	2	1	4	7 (2.63%)
2+	9	9	1	3	22 (8.27%)
3+	2	1	1	2	6 (2.25%)
4+	0	1	2	0	3 (1.12%)
>4	0	0	1	0	1 (0.37%)
Total	69	75	53	69	266

prevalence in the region[18]. In our study this was not observed, and that the highest rates of detection occurred after 40 years of age. In other studies in Brazil the mean age at diagnosis was 49 yrs and 2,2% were children [19]. This fact leads us to reflect that leprosy is not endemic in Cristalina city. Leprosy is most prevalent among the less affluent population[20], which is in accordance with our study, where it was shown that rural areas of the city had the highest detection rates. The growing number of cases does not seem to be linked with the expansion of the disease, but with greater monitoring and control measures against the disease, which allowed an increase in the number of diagnoses. This may be compared to a study conducted in northeastern Brazil, where the low detection rates is an apparent underreporting of the cases [16]. The bacillary index of zero represents the negative cases for bacilloscopy, and can not infer the presence or absence of the disease. Clinical and other examinations are necessary to establish the diagnosis. The present study is not aimed to classify or establish diagnosis, but raise the importance of bacilloscopy as a tool to aid diagnosis. Nevertheless, it is known that cases are being diagnosed late (64.10% after 40 years of age), with predominance among males; added to this, 227 (85.33%) were paucibacillary or negative/false negative cases. These cases were not confirmed by bacilloscopy which makes it impossible to show the realities of the study population.

Leprosy could be classified based on the number of skin lesions: where Paucibacillary (PB) leprosy corresponds with 1-5 skin lesions (indeterminate leprosy, TT and BT) and MB leprosy with >6 skin lesions (BB, BL and LL) [21].However, patients may be misclassified with risk of both over and under treatment. Therefore, to avoid this misclassification, experts recommend classifying patients according to the Ridley–Jopling scheme. Skin smears is used to assess the bacillary load: BI < 2 are designated as PB while a BI > 2 as MB [21].Although, the WHO recommended the method of counting skin lesions to classify a patient as PB or MB, a BI method shows poor sensitivity in PB cases, but their high specificity is very useful for identifying MB, which is the most infectious. The BI method used in Cristalina can be used as an alternative for diagnosis, treatment and hence decrease the transmission of new cases of leprosy. Bacilloscopy and the BI have an importance in leprosy eradication because patients that had a high BI could still have a positive BI after the end of the treatment, and the total clearance of residual bacilli can take years [22].

The finding that the majority of all diagnosed cases are paucibacillary reinforces the importance of early diagnosis and treatment considering that a delay in diagnosis predisposes the patient to disabilities. In the present study, it should be noted that the bacilloscopy was very important in the diagnosis, and indirectly in the clinical classification of different cases. This technique is important for leprosy eradication in Cristalina, Brazil because it has a low cost and can be deployed with greater accessibility in developing countries. The observation reported here provides evidences to health managers for improving actions for Leprosy Control in order to achieve the goal of elimination. The active search for new cases is an important method for disease control.

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