Revista da Sociedade Brasileira de Medicina Tropical
17:179-186, Out-Dez, 1984

HUMAN MUCOCUTANEOUS LEISHMANIASIS IN TRÊS BRAÇOS, BAHIA – BRAZIL. AN AREA OF *LEISHMANIA BRAZILIENSIS BRAZILIENSIS* TRANSMISSION. III. MUCOSAL DISEASE PRESENTATION AND INITIAL EVOLUTION

Philip D. Marsden[1], Elmer A. Llanos-Cuentas[1], Edinaldo L. Lago[2], César C. Cuba[1], Air C. Barreto[1], Jackson M. Costa and Thomas C. Jones[3]

In an analysis of 57 patients mucosal disease was commonest in males (77%) in the third decade of life although the age range was wide and even two children were affected. All but nine patients (16%) had signs of cutaneous leishmaniasis but in only 8(14%) was this lesion active. The nose was affected in 100% of 19 patients with multiple lesions and 92% of 38 patients with single lesions. The pharynx, palate, larynx and upper lip were affected in this order: 42% of patients with multiple lesions had laryngeal disease and in two patients this site existed as a lone lesion. No age difference could be discerned as to whether lesions were single or multiple. Duration of mucosal disease was very variable from less than 4 months to 264 months. Only 7% developed mucosal disease more than ten years after the cutaneous lesion.

Usually patients responded to adequate antimonial treatment but there were exceptions, when amphotericin B had to be used. Three patients who refused to collaborate regarding treatment died. Only 18% of patients in whom measurements were made had positive fluorescent antibodies two years after treatment.

Key words: *Leishmania braziliensis braziliensis*. Mucosal leishmaniasis. Clinical presentation. Evolution. Treatment.

It is the mucosal lesions that give most cause for concern in human *Leishmania braziliensis braziliensis* infections. The difficulty in treating this condition is notorious and our experience in Três Braços confirms this fact. It is in this group that often prolonged hospital admission has been necessary to halt the progress of multilating disease. Also amphotericin B had to be used in some patients when antimony treatment failed. Both in the hospital and the field all the deaths we can attribute to this disease over the years have occurred in this group. Death is usually due to a complex of factors, secondary malnutrition due to difficulty in swallowing, secondary infection of the lungs due to aspiration of infected secretions or suffocation due to closure of the laryngeal aperture.

MATERIAL AND METHODS

We have previously described the diagnostic methods and routine procedures adopted for the treatment of leishmaniasis in Três Braços[7]. Mucosal lesions were recorded on a diagram of the nose throat and larynx, scars of cutaneous infections were described and measured and a previous history of cutaneous infection obtained. Granuloma was biopsed using a cutting biopsy punch. The techniques for histology and parasite isolation are as described in the first paper in this series[7]. Patients were classified as to whether a single mucosal surface or multiple structures were affected.

If the patient had never received adequate antimony treatment and someone was available to give and check injections he was initially treated in the area. Three series of ten days each to a total dose of glucantime of 1 gram of drug per kilo body weight per series was used[1]. This is equivalent to a daily dose of 28 mg Sb^V per kilogram body weight. If response was poor and mucosal granuloma persisted we advised the patient be admitted to our hospital in Brasília.

1. Núcleo de Medicina Tropical e Nutrição, University of Brasília, Brazil.
2. SUCAM, Ministry of Health Brazil.
3. Cornell Medical Center, New York.

This work was supported by the Brazilian Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – Grants No. 403682/82 and 403690/82), The Brazilian Ministry of Health (SUCAM), UNDP/World Bank/W.H.O. Special Programme for Research and Training in Tropical Diseases and a United States Public Service grant (AI 16282-04) administered by the Department of International Medicine, Cornell Medical College, New York.
Recebido para publicação em 16/8/84.

In Brasília either more extensive antimonial treatment was used or where deemed necessary amphotericin B. Follow up was accomplished by periodic visits to the patient in his home. In July 1983 when this analysis ended all but three patients had been followed for a minimum of six months after treatment. The mean follow up time was 33 ± 26 months. Observations over periods greater than 1 year were available in 73%, two years in 47% and five years in 21% of patients.

During the period of active disease they were usually seen at the end of each treatment series, at six months and then yearly. At these follow up examinations the nose and throat were examined with a good light, a nasal speculum and a tongue depressor. After a throat anaesthetic spray the larynx was examined using a laryngeal mirror. The naso pharynx and back of the nose was not visualised. If no evidence of activity was seen only a serological specimen for IFA was obtained. Evolution was measured by scarring of the initial lesion without recurrence (clinical cure) and serial indirect fluorescent antibody tests.

RESULTS

Fifty-seven patients with mucosal leishmaniasis were seen at our field clinic between July 1976 and July 1983. Isolates from the lesion of eleven patients in this group were identified: all were *L. braziliensis braziliensis*.

Nine (16%) of the 57 patients had no history of a skin lesion or sign of a scar. The characteristic depressed hypopigmented scar of past cutaneous disease was present in 40 patients (70%). Eight patients (14%) had an active skin lesion. In three patients after healing of a primary skin lesion 1, 2 and 8 years later, a mucosal lesion developed which was accompanied by a further active skin lesion.

Table 1 shows the distribution of 57 patients in relation to age compared to the distribution of patients developing primary cutaneous lesions. 68% of patients with mucosal disease were between 20 – 60 years old, the most being between 20 – 29 years old. The oldest was 73 and the youngest 8 years old. The high age of onset (over age 20 in 82% of cases) differs from the age of the primary lesion in which only 40% were over 20 years old[12]. No age difference was discerned as to whether lesions were single or multiple. 77% were male ($p < 0.001$).

Table 1 – *Age of patients presenting with mucosal disease compared to those presenting with primary cutaneous lesions in Três Braços.*

Age in years	*Mucosal*		*Primary cutaneous**
	Nº	*%*	*%*
0 – 9	2	3.5	20.3
10 – 19	8	14.1	39.6
20 – 29	18	31.6	9.9
30 – 39	9	15.7	7.2
40 – 49	9	15.7	11.6
50 – 59	3	5.3	7.6
> 60	8	14.1	3.8
Total	57	100.0	100.0

(*) Data from reference 12.

Table 2 – *Distribution of 57 patients with mucosal leishmaniasis as regards the number and site of lesions.*

	Single lesion		*Multiple lesions*	
Site	*Nº*	*%*	*Nº*	*%*
Nose	35	92.1	19	100.0
Pharynx	–	–	18	94.7
Palate	1	2.6	16	84.2
Larynx	2	5.3	8	42.1
Upper lip	–	–	6	35.1
Number of patients examined	38	100.0	19	

The mucosal area most affected was the nose (100% of multiple lesions and 92% of single lesions (Table 2). The pharynx, palate, larynx and upper lip were affected in this order of frequency. 42% of patients with multiple lesions had laryngeal disease and in two patients this site existed as a lone lesion. Early symptoms were epistaxis, persistent mucosal secretion, intermittent blockage and discharge of granuloma tissue on occasion. Smell was not impaired but many patients complained of nasal irritation. At times differentation from allergic rhinitis was difficult and the diagnosis could only be made when a granuloma was visualised.

Table 3 – Distribution of 55 patients with mucosal leishmaniasis as regards duration of disease and number of lesions (*)

Duration of disease (months)	*Single lesion*		*Multiple lesions*		*Total*	*%*
	N°	*%*	*N°*	*%*		
12 or less	10	27,8	8	42,1	18	32,7
13 – 24	11	30,6	3	15,8	14	25,5
25 – 60	6	16,6	3	15,8	9	16,4
61 – 120	6	16,6	4	21,0	10	18,2
121 or more	3	8,4	1	5,3	4	7,2
Total	36	100,0	19	100,0	55	100,0

(*) Two patients could give no reliable history.

Fifty-five patients could remember the duration of their disease. This information is summarised in Table 3. The duration of disease was very variable and was similar in single and multiple lesions. Relatively minor nasal granulomas were seen in patients with a history of more than ten years. In 31% the history was under a year, in 56% under two years and in 7% over ten years. The long duration of disease indicates the prolonged period of absent medical care in the region, since in our recent experience this interval has been shortened considerably. It is presented here to emphasise the fact that prolonged, non-progressive mucosal disease can be observed.

Table 4 – *Distribution of 35 patients with mucosal leishmaniasis as regards the time interval between scarring of the primary lesion and initiation of the mucosal lesion.*

Time interval in years	*Number of patient*	*%*
1 or less	9	25,7
1 – 2	7	20,0
3 – 5	6	17,2
5 – 10	9	25,7
10 – 15	2	5,7
more than 15	2	5,7
Total	35	100,0

Among the 35 patients who could give an accurate history of the primary lesion the time interval between healing of the skin lesions and the onset of mucosal disease was also variable (Table 4). In 46% of patients this period was less than two years but it varied greatly from six months to thirty years. The mean time was 3 – 4 years.

Inadequate treatment with consequent delay in healing of the primary skin lesion seems a highly significant risk factor in the development of mucosal disease. The history regarding previous treatment of these patients was as follows: 29 had previous treatment principally with glucantime but also other anti-leishmanial drugs such as trivalent antimonials and nifurtimox, 19 insisted they had no treatment whatever and nine could not remember. Of the 23 patients who received glucantime only one had received sufficient total dose by our criteria[12] and only four had taken regular therapy. Many patients had only used one ampoule of glucantime at 3 – 7 day intervals and generally only 10 – 12 ampoules as the total dose. Only one patient in our experience has developed mucosal disease after a regular adequate dose of glucantime for his previous cutaneous lesion. We have seen three patients develop mucosal disease after treatment for cutaneous disease with nifurtimox; the only drug available at the time.

Prospectively we were able to study 22 patients (13 with single lesions and 9 with multiple lesions) as regards the influence of regular treatment on clinical cure. Fifteen patients who used the treatment as prescribed achieved clinical cure in 4.2 ± 1.3 months. This is significantly less time than the seven patients in whom treatment was irregular (9.5 ± 3.5 months, $p < 0.001$). There was no significant difference in healing time in patients with single or multiple lesions.

Fourteen patients failed to respond to our regular glucantime therapy. These were either given further glucantime therapy or hospitalised and treated with amphotericin B. One eight year old girl (LTB 133)

developed mucosal lesions during glucantime treatment for her skin leishmaniasis (Fig. 1). Six patients responded well to amphotericin and have been followed without relapse for periods ranging from 10-72 months. One patient refused all further intravenous treatment after taking less than one gram of this drug as a total dose. The lesion recurred and patient was subsequently cured at home with prolonged nifurtimox therapy[8] and has been followed for 65 months without relapse. A further patient with a six year history resistant to nifurtimox and antimony treatment relapsed after 2.5 grams of amphotericin and still had active buccal granuloma and recoverable *L. b. braziliensis* at the end of the study (Figure 2).

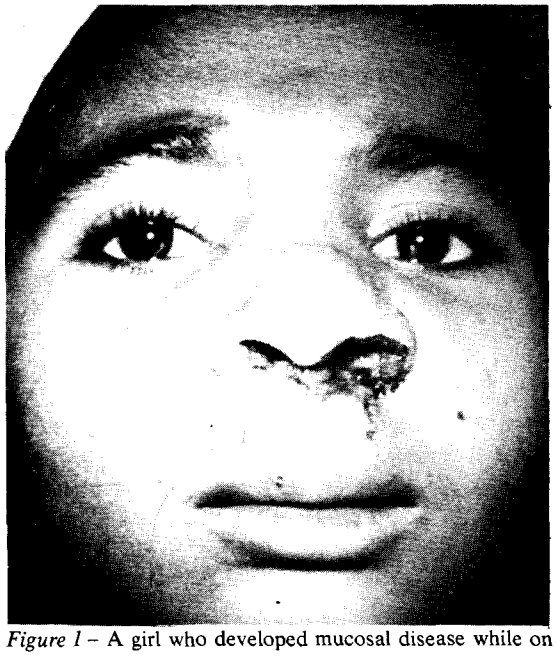

Figure 1 – A girl who developed mucosal disease while on glucantime therapy. Cured with amphotericin B (LTB 133).

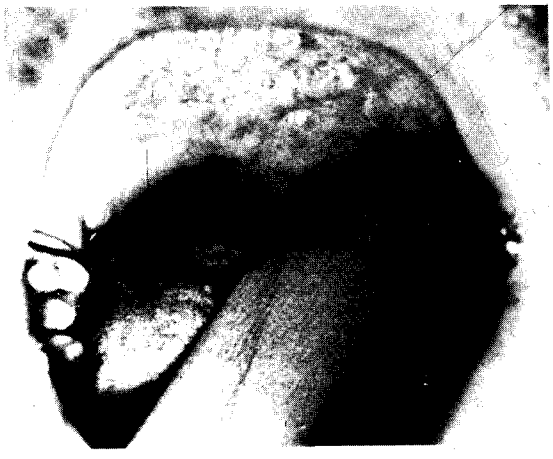

Figure 2 – Lesion only involving the hard and soft palate remaining active after eight years despite repeated antimony treatment (glucantime and pentostam) and 2.5 grams total dose of amphotericin B (LTB 12).

Figure 3 – A patient with a ten year history of multiple site mucosal disease. Cured with amphotericin B (LTB 208).

Figure 4 – Multiple site mucosal disease involving the nose, palate and pharynx resulting in facial lymphoedema. Although the mucosae are inactive the lymphoedema has only resolved partially after amphotericin B (LTB 259).

Data were available on the indirect fluorescent antibody test during, immediately after treatment and up to two years later in 31 patients. Only one patient did not have a significant titre (> 20). One year after treatment only 42% of patients had a significant titre and only 17% two years later. In patients who failed on treatment IFA titres were maintained.

The commonest complication of mucosal disease was perforation of the nasal septum which occurred in 55% of patients with a single lesion and 58% of patients with multiple mucosal lesions. Amputation of the cartilaginous nose occurred in 7% and 16% of patients with multiple lesions were left with tissue defects of the face. Laryngeal disease was associated with sialorrhea and even after successful treatment a degree of dysphonia always persisted.

The three deaths due to leishmaniasis in this study (5%) all occurred in patients with multiple lesions who refused hospital admission and did not collaborate with their treatment schedules for various reasons (stubborness, senile dementia, alcoholism). All three developed terminal respiratory infections due to aspiration of infected secretion and two had considerable upper airway obstruction as a terminal event.

DISCUSSION

There is no value in trying to deduce prevalence or incidence figures from our data. Such a clinic series is selected in favour of patients with severe disease and therapeutic problems. Our initial survey in Três Braços recorded a 30% prevalence of mucosal disease[4] but as we emphasised then this high rate is due to the accumulation of such patients. In the absence of treatment facilities they do not get better. Our success in resolving the problems of this group has led to other patients appearing, sometimes travelling for days to reach our clinic. Recently from our unpublished data of a four year prospective field study of 1879 people living in fifteen farms around Três Braços the prevalence of mucosal disease among at risk primary lesion cases has been calculated at 3.2% (Jones TC: personal communication).

As regards age and sex distribution our small series is similar to the 1,791 cases analysed in 1936 by Barbosa in São Paulo[3]. The distribution of mucosae affected in the two series is also similar except that our series contains a proportionately greater number of laryngeal cases.

Most patients were in third decade of life and historical data regarding onset (Table 3) suggest that in the majority of patients the relapse of the infection in the form of mucosal granuloma occurs relatively soon after cutaneous infection confirming the observations of Villela[19]. However we too have seen patients in the area where relapse has only occurred over 10 years later [17 20]. It could be argued that in an unknown number of patients the mucosal lesion represent a fresh infection. This seems to us unlikely since most patients give a clear history of previous skin infection and have characteristic skin scars. Where such findings are not present the initial skin infection may have been so fleeting as to not leave a permanent scar or to have been remarked by the patient. Phlebotomines actually biting the facial mucosa seems a rare event. A few patients develop mucosal lesions while the skin lesion is still active. Scrutinising individual case records we cannot explain why there is great variation in the time of appearance of mucosal lesions.

That 77% were male is noteworthy in view of the equal sex ratio found in cutaneous disease[12]. The most likely explanation is that more males were exposed in Três Braços to infection risk in the past[4]. For economic reasons in recent years many women now work on the farms; accounting for a change in sex frequency in our data on cutaneous disease[4 12]. It raises the question of what host factors determine the development of mucosal disease. A high proportion of our patients seem to be of a darker colour than is average in the population although this is still at the level of a clinical impression. Racial differences in the incidence of mucosal disease has been suggested in Bolivian patients[21]. We have detected two families with what seems to be an unusually high incidence of mucosal disease (Marsden et al: unpublished observations). Although there is strong evidence of host determination of disease expression in inbred mice[5] the relevance of this to man is speculation but obviously investigation of the genetic constitution of our patients with mucosal involvement would be worthwhile.

Single lesions were almost always in the nose. Kanan and Ryan[9] have evidence that venules in the nasal septum permit particulate matter to escape into the tissues and this could be the case in blood borne Leishmania. They also raise the interesting possibility that the localisation of leishmanial granuloma to the upper respiratory and alimentary passages is related to the cold air current passing the mucosa and resulting in more favourable temperatures. Certainly Leishmania are known to be markedly thermosensitive[24]. However although the septum is a privileged site[10] these

suggestions are difficult to fit into the pattern of the disease in our 57 patients. Why the larynx as a lone lesion where surely a temperature effect must be minimised? Why are lesions of the upper lip relatively rare? Lymphatic drainage from the anterior nose to the pharynx and palate is minimal and yet the second commonest lesion is there. Could trauma play a role here?

Nasal lesions were principally of the anterior septum a fact borne out by the high incidence of septal perforation. However we have a difficulty in examining the posterior nasal space and it could be we have missed granuloma high in the nose. Yet the fact that many patients with extensive disease of the mouth and throat can still breath freely through both nostrils suggests that granulomas do not arise by contiguous spread. It appears that those in the nose, mouth, throat and larynx are usually separate events. That the onset of multiple lesions occurs as early as single lesions suggests an underlying host factor as a determinant rather than simply parasite multiplication and contiguous spread.

Factors relating to the nature of the primary cutaneous infection play a significant role in the risk of developing mucosal disease[11]. The number of skin lesions and their size and also whether they occur above the belt are all potentially dangerous factors. In anatomical terms this makes sense since more larger lesions will have more parasites with a increased risk of metastasis. Also lymphatic drainage routes up which amastigotes must course to gain the circulation will be shorter with fewer lymph gland filters than those say on the leg[23]. Our failure to date to isolate circulating Leishmania in such patients could be because our techniques of culture and hamster inoculation are not sufficiently sensitive.

Bryceson[6] has speculated that the induction of immune response in lymph glands preoccupied with other antigens could lead to a state of tolerance and permit metastasis. Certainly removal of the draining lymph gland in the mouse model leads to disease excerbation[15]. Both these observations could have relevance to mucosal leishmaniasis which is, after all; a metastasis. However the fact that this entity occurs almost exclusively in *L. b. braziliensis* infections suggests we must look also at the properties of this specific parasite as regards its capacity for distant spread from the site of the lesion. At the moment no suitable animal model exists.

Both inadequate treatment and a prolonged scarring time had a highly significant correlation with the occurrence of mucosal disease. Obviously one correlates with the other and reinforces the view that maximum antimonial therapy is desirable in patients at particular risk. If toxicity studies permit its use it would obviously be desirable to give 30 days continuous therapy at a level of 20mg Sb^V per kilo body weight[18]. Quite rightly dosage schedules are to be preferred expressed in this more accurate manner than as grams of glucantime per kilo per series as we have done in the past[2]. However three interrupted schedules of glucantime properly given have had good results in our hands even in mucosal disease. We are now trying shorter more intensive schedules of glucantime. It is significant that most of our relapses occurred when we were using nifurtimox (Lampit) in the area. We have already shown this to be a weak antileishmanial agent inadequate for mucosal disease[8 14].

As our experience of glucantime has grown its value in adequate doses has become clearer. Our early observations suggested glucantime was relatively ineffective in some patients[16] but, by today's standards, they were not using glucantime in sufficient dose for a long enough period. Amphotericin B is of great value in patients who do not respond to pentavalent antimonials but as our results show such patients are rare. Recently we are using amphotericin in fewer patients because, as suggested by Bryceson (personal communication), if sufficient pentavalent antimony is given cure is achieved. Our one patient in this analysis who relapsed after 2.5 grams total dose of amphotericin (LTB 12) is a good example. He had received much previous antimonial treatment including a thirty day continuous course of pentostam but subsequently he has been cured by 85 days continuous antimony therapy (dose 20 mg Sb^V/kilo body weight per day).

The difficulty in evaluating cure in mucosal leishmaniasis is related to our difficulties in isolating the organism and visualising granuloma sites. Certainly longitudinal follow up for years is advisable and this programme continues in Três Braços. Regular clinical examination over time is still the best criterion of cure[1]. Whether the parasites persists in such patients is speculative. As reported by Walton[22] in the majority of cases antibody titres fall slowly after clinical cure. We are still following the patients in this series with residual positive serology.

We have already reported a death in a patient who failed to respond to both these drug options[13].

Our three deaths in patients with multiple lesions in this series were all complicated by factors of failure to agree to the treatment prescribed. In spite of the residual mutilation we have repeated evidence that we can restore the great majority of these patients to an active social life and eventually, when serology has been negative for at least a year, consider plastic surgery.

RESUMO

Numa análise de 57 pacientes o acometimento da mucosa foi mais comumente observado em homens (77%) na terceira década de vida, embora fosse grande a variação das idades e ocorrendo mesmo o acometimento de duas crianças. Com a exceção de nove pacientes (16%) todos os outros tinham sinais de leishmaniose cutânea sendo que em somente oito (14%) de lesão era ativa. O acometimento do nariz foi observado em 100% de 19 pacientes que apresentavam lesões múltiplas e em 92% de 38 pacientes apresentando uma única lesão. A faringe, palato, laringe e lábio superior foram afetados nesta experiência. 42% dos pacientes com lesões múltiplas apresentavam acometimento da laringe sendo que em dois pacientes a única lesão existente apresentava-se neste ponto. Não foi observada qualquer diferença relacionada com a idade no que se referia à existencia de lesões únicas ou múltiplas.

A duração do acometimento da mucosa variou de menos de 4 até 264 meses. Somente 7% desenvolveram o acometimento da mucosa após mais de dez anos o desenvolvimento da lesão cutânea.

Os pacientes usualmente responderam ao tratamento adequado por antimonial embora em algumas exceções fosse usada amphotericina B. Morreram três pacientes que se recusaram a colaborar no tratamento.

Dois anos após o tratamento observou-se positividade de anticorpos fluorescentes em somente 18% dos pacientes entre aqueles acompanhados.

Palavras chaves: *Leishmania braziliensis braziliensis*. Leishmaniose de mucosa. Apresentação clínica. Evolução. Tratamento.

REFERENCES

1. Anonymous. Report of the workshop on the chemotherapy of mucocutaneous leishmaniasis. World Health Organization TDR/Leish/MCL/79 3, 1979.

2 Anonymous. Report of the informal meeting on the chemotherapy of visceral leishmaniasis. World Health Organization TDR/Chem. Leish/VL 82 3, 1982.

3. Barbosa JER. Dados estatísticos sobre os casos de leishmaniose das mucosas observados no Serviço de Otorrinolaringologia da Santa Casa de São Paulo. Revista Otorrinolaringológica de São Paulo 4: 697-714, 1936.

4. Barreto AC, Cuba CC, Marsden PD, Vexenat JA, De Belder M. Características epidemiológicas da leishmaniose tegumentar americana em uma região endêmica do Estado da Bahia, Brasil. I. Leishmaniose humana. Boletin de la Oficina Sanitaria Panamericana 90: 415-424, 1981.

5. Bradley DL. Genetics of resistance to infection with special reference to leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene 76: 143-146, 1982.

6. Bryceson ADM. Diffuse cutaneous leishmaniasis in Ethiopia. II Treatment. Transactions of the Royal Society of Tropical Medicine and Hygiene 64: 369-379, 1970.

7. Cuba CC, Barreto AC, Llanos-Cuentas AE, Magalhães I, Lago EL, Reed SG, Marsden PD. Human mucocutaneous leishmaniasis in Três Braços, Bahia – Brazil. An area of *Leishmania braziliensis braziliensis* transmission. I Laboratory diagnosis. Revista da Sociedade Brasileira de Medicina Tropical 17: 161-167, 1984.

8. Guerra M, Marsden PD, Cuba CC, Barreto AC. Further studies on nifurtimox in the treatment of mucocutaneous leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene 75: 335-337, 1981.

9. Kanan MW, Ryan TJ. The localisation of granulomatous diseases and vasculitis in the nasal mucosa. In: Vasculitis Stasis and Ischaemia p. 195-220. WB Saunders, Philadelphia, 1975.

10. Klotz O, Lindenberg H. Pathology of leishmaniasis of the nose. American Journal of Tropical Medicine 3: 117-141, 1923.

11. Llanos-Cuentas EA, Marsden PD, Cuba CC, Barreto AC, Campos M. Possible risk factors in development of mucosal lesions in leishmaniasis. Lancet 2: 295, 1984.

12. Llanos-Cuentas AE, Marsden PD, Lago EL, Barreto AC, Cuba CC, Johnson W. Human mucocutaneous leishmaniasis in Três Braços, Bahia – Brazil. An area of *Leishmania braziliensis braziliensis* transmission. II Cutaneous disease. Presentation and evolution. Revista da Sociedade Brasileira de Medicina Tropical 17: 169-177, 1984.

13. Marsden PD, Sampaio RNR, Rocha R, Radke M. Mucocutaneous leishmaniasis – an unsolved clinical problem. Tropical Doctor 83: 131-139, 1977.

14. Marsden PD, Cuba CC, Sampaio RN, Rocha R, Barreto AC. Nifurtimox in the treatment of mucocutaneous leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene 73: 391-394, 1979.

15. Poulter IW, Randolph CR. Mechanisms of immunity in leishmaniasis. IV Significance of lymphatic drainage from the site of infection. Clinical and Experimental Immunology 48: 396-402, 1982.

16. Rocha RAA, Sampaio RN, Guerra M, Magalhães I, Cuba CC, Barreto AC, Marsden PD. Apparent glucantime failure in five patients with mucocutaneous leishmaniasis. The Journal of Tropical Medicine e Hygiene 83: 131-139, 1980.

17. Sampaio RNR, Rocha RAA, Marsden PD, Cuba CC, Barreto AC. Leishmaniose tegumentar americana. Casuística do Hospital Escola da UnB. Anais Brasileiro de Dermatologia 55: 69-76, 1980.

18. Veiga JPR, Wolff ER, Sampaio RN, Marsden PD. Renal tubular dysfunction in patients with mucocutaneous leishmaniasis treated with pentavalent antimonials. Lancet, 2. 569. 1983.

19. Villela F. Dados estatísticos sobre a leishmaniose das mucosas em Araçatuba, São Paulo. Folha Médica do Rio de Janeiro 20: 243-244, 1939.

20. Walton BC, Chinel LV, Equia OE. Onset of espundia after many years of occult infection with *Leishmania braziliensis*. The American Journal of Tropical Medicine e Hygiene 22: 696-698, 1973.

21. Walton BC, Valverde L. Racial differences in espudia. Annals of Tropical Medicine and Parasitology 73: 23-29, 1979.

22. Walton BC. Evaluation of chemotherapy of American leishmaniasis by the indirect fluorescent antibody test. The American Journal of Tropical Medicine e Hygiene 29: 747-752, 1980.

23. Wilson HR, Dieckmann BS, Childs GE. *Leishmania braziliensis* and *Leishmania mexicana:* experimental cutaneous infections in golden hamsters. Experimental Parasitology 47: 270-283, 1979.

24. Zeledón R. Efecto de la temperatura de la piel en la leishmaniasis cutanea experimental. Revista da Sociedade Brasileira de Medicina Tropical 5: 131-134, 1971.