Association between leprosy reactions after treatment and bacterial load evaluated using anti PGL-I serology and bacilloscopy

Associação entre reação hansênica após alta e a carga bacilar avaliada utilizando sorologia anti PGL-I e baciloscopia

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ABSTRACT

Leprosy (Hansen's disease, HD) reactions are immune-inflammatory phenomena that occur during the evolution of the disease. Given the current criteria for treatment of the disease, this event is often observed after the patient has been released from treatment (RFT) from multi-drug therapy (MDT). A case-control study was conducted comparing laboratory results of cases of leprosy reactions after RFT from multibacillary MDT (MDT/MB) with a control group to analyze the possible association between posttreatment reactions and bacterial load using the ML Flow serological test for detection of antibodies to *Mycobacterium leprae* and the results of bacilloscopic skin smears. The study was conducted in two reference centers in Recife, Pernambuco State, Brazil, involving 208 patients. The results obtained indicate that posttreatment reaction is statistically associated with bacterial load through positive serology post-RFT. In conclusion, common risk factors exist between relapses and post-RFT reactions.

Key-words: Hansen's disease (leprosy). ML Flow serological test. Leprosy reactions.

RESUMO

As reações hansênicas são fenômenos imuno inflamatórios que ocorrem durante a evolução da hanseníase. Atualmente com os critérios de finalização de tratamento esta intercorrência pode ser observada após a alta da poliquimioterapia. Trata-se de um estudo caso-controle onde foram comparados, laboratorialmente, os casos de reação hansênica após alta da poliquimioterapia multibacilar (PQT/MB) com o grupo controle para analisar a possível associação entre a reação hansênica após alta e a carga bacilar, utilizando o ML Flow, teste sorológico para detecção de anticorpos contra o *Mycobacterium leprae,* e os resultados das baciloscopias cutâneas. O estudo foi realizado em dois serviços de referência na Cidade de Recife – Pernambuco – Brasil, onde participaram 208 pacientes. Os resultados encontrados indicam que a reação após alta está estatisticamente associada à carga bacilar através da positividade do teste sorológico após alta. Conclui-se que existem fatores de riscos comuns entre a recidiva e a reação após alta.

Palavras-chaves: Hanseníase. Teste sorológico ML Flow. Reações hansênicas.

In the last 15 years, several studies have been conducted that have sought to evaluate the evolution of leprosy (Hansen's disease, HD) in both multibacillary and paucibacillary cases over the course of multi-drug therapy (MDT)[13 21]. In general, these analyses looked at the clinical and bacteriological evolution of these individuals and the incidence and characteristics of leprosy reactions, principally during the treatment period, with rare analysis of the posttreatment phase.

The development of MDT provided the prospect of disease cure in a shorter timeframe, the possibility of elimination of the disease as a public health problem and increased attention focused on the occurrence of leprosy reactions[13].

Two basic types of leprosy reactions are currently recognized[1]. The reversal reaction (RR) or type 1 reaction, related to an increase in cellular immunity, which occurs primarily in patients with the borderline tuberculoid (BT), borderline borderline (BB) and borderline lepromatous (BL) forms. The second type is traditionally known as erythema nodosum leprosum (ENL) or type 2 reaction, which is commonly observed in patients with the lepromatous (LL) and borderline lepromatous (BL) forms and is related to the exacerbation of humoral immunity. Observation of leprosy reactions involving pain and the thickening of nerve stems not association with the cutaneous symptoms of a type 1 or type 2 reaction are also possible, which some authors consider a third type of reaction called isolated neuritis[11].

Certain conditions tend to lead to reactional episodes: intercurrent diseases, pregnancy, childbirth and physical or emotional stress[6]. These are largely preventable factors, but in the vast majority of reactions, their appearance and control are

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directly related to the immunological instability of the patient. However, when these take place after release from treatment (RFT), they can be confused with HD relapse. For this reason, research is necessary to determine clinical markers and laboratory tests that are sensitive, precise, practical and economical to identify risk factors and facilitate differential diagnosis of these two scenarios.

Phenolic glycolipid-I (PGL-I) is a specific surface antigen of the cellular wall of *Mycobacterium leprae* and an elevated number of specific antibodies against this antigen reflects the activation of a humoral response and the presence of a high bacterial load. Associations have been made with the presence of immunoglobulin M (IgM) against PGL-I and the clinical form of Hansen's disease, treatment monitoring and the risk of relapse[4,5]. In addition, associations exist between the detection of subclinical infection in household contacts and the risk of onset of the disease[16,19], but there are a limited number of references involving the use of PGL-I with posttreatment reactions[20].

In the pursuit of data to determine clinical and laboratorial markers for reactions after release from treatment, a study was conducted to evaluate the association between these types of reactions and the bacterial load of MB patients using PGL-I serology and bacilloscopy as markers.

MATERIALS AND METHODS

Study design and population

A case-control study was conducted involving 208 patients, 104 of whom presented reactions after having completed the MDT regimen and who were recruited at the moment of reaction diagnosis (cases) and 104 patients with no posttreatment reactions (controls), matched by year of RFT with a margin of ± 6 months from the end of treatment. The bacterial load was considered the main exposure, using PGL-I serology and bacilloscopy as its markers.

All patients were treated with MDT/MB and monitored in the dermatology out-patient clinic of the Amauri de Medeiros Integrated Health Center (CISAM) of the University of Pernambuco (UPE) and in the dermatology department of the Federal University of Pernambuco (URPE) Clinical Hospital.

Criteria for inclusion

- Patients classified as MB at the time of diagnosis using clinical criteria and/or an initial bacteriological index (IBI) >0, as well as determinations made based on the number of skin lesions and treated with MDT/MB, as standardized by the World Health Organization (WHO) and adopted by the Brazilian Ministry of Health (MoH), with 12 or 24 doses
- Patients (case group) with reversal reactions (RR) presenting with or without neuritis (type 1 leprosy reaction) and/or Erythema Nodosum Leprosum (ENL) with or without neuritis (type 2 leprosy reaction), as well as patients with isolated neuritis. The clinical criteria for diagnosis of RR (type 1 reaction) were the appearance of new lesions or preexisting lesions with erythema/infiltration, with or without thickening or pain in peripheral nerves (neuritis) with or without edema in the hands, feet or face. For ENL (type 2 reaction), sudden onset of painful nodules and erythematous or purple coloration, possibly showing blistering or ulceration, fever and/or neuritis.

Definition of neuritis

A diagnosis of neuritis was made when the following criteria were observed:

- Pain, either spontaneous or upon palpation, in the nerve, accompanied by thickening of the nerve stem;
- Recent loss of sensitivity or motor function (< 6 months in duration).

Laboratory exams

Results from lymph smear microscopy using Ziehl & Neelsen staining determined the bacteriological (BI) and morphological (MI) indexes of patients as per routine examination. Evaluation of the BI was determined at two points in time: before treatment (IBI) and as part of the study (IB), after having been released from treatment.

Once a post-RFT reactional episode occurred, all patients provided a sample of total blood in order to perform the ML Flow serological test to detect IgM against the PGL-I of *Mycobacterium leprae*. The exam was completed as described previously by Bührer-Sékula *et al*[5]. The result is positive when the test line turns colored and positivity is quantified as 1+, 2+, 3+ and 4+, considering the intensity of test line coloration. A negative result is indicated by the absence of a line on the test strip.

Statistical analysis

The association between the dependent variable and each of the independent variables considered in the study was verified using: calculation of odds ratio (OR) for paired data, confidence interval (CI 95%) and p-value.

The level of statistical significance, determined by the rejection of the null hypothesis, adopted in all statistical analyses was a maximum error of 5% (type I error) ($p<0.05\%$) when the aforementioned associations were investigated. In the multivariate analysis, the conditional logistic regression model was composed of variables which in the univariate analysis presented $p \leq 0.20$ in the association with post-MDT treatment reaction. The multivariate analysis was conducted for each block of variables that were adjusted for each other and for the ML Flow result.

This study was approved by the Research Ethics Committee of the Federal University of Pernambuco under protocol no. 252/2004. All of the patients involved agreed to participate and signed a declaration of free informed consent.

RESULTS

Of the 208 patients studied, 65.8% were men; patient mean age was 41.08 years-old (SD ± 16.81) and the average length of time after RFT was 46.47 months (SD ± 34.73). The average

period of time elapsed from the end of MDT to the appearance of reactions was 14.12 months (SD± 28.89), with a majority (78.7%) presenting reactions in the first 12 months after RFT and 6.8% more than 5 years after RFT (data not presented).

In relation to the sex of the patient, an association occurred between the appearance of reactions after RFT, with men showing a 2.07-fold greater chance of suffering posttreatment reactions (p= 0.025). This result did not remain significant in the multivariate analysis. Distribution by age group showed that patients over 60 years of age were 4 times less likely to develop reactions after RFT than the control group.

Regarding the clinical form of the disease, a statistically significant association (OR = 3.7) occurred between those with posttreatment reactions. Lepromatous patients had a 3.7-fold greater chance of developing reactions after RFT than patients with the borderline form.

As for the presence of reactional episodes during treatment, 59.6% of the patients studied (124/208) had suffered these episodes. This association between reactions during and after treatment was statistically significant (p= 0.00), where the patients that had reactions while undergoing MDT/MB also had a 4.33-fold greater likelihood of having them after treatment.

The predominant type of reaction during treatment among the cases was type 2 (56.4%), followed by type 1 (25.6%) and isolated neuritis (18%), whereas in the control group, type 1 reactions were more prevalent (52%) followed by isolated neuritis (30.4%) and type 2 reactions (17.4%). The patients with type 2 reactions (ENL) during treatment had a 4.34-fold greater chance of developing post-RFT reactions than those with other types of reactions.

The IBIs were associated with posttreatment reactions, such that patients with an IBI > 2.0 presented a higher chance of developing reactions than the control group (p = 0.000).

At the time of the study, the BI+ cases had a 7.21-fold greater chance of developing posttreatment reactions than the control group (p = 0.00).

The patients with a PGL-I positive serology (ML Flow) after MDT presented a 10.4-fold greater chance of developing posttreatment reactions (p = 0.00) than the control group (**Table 1**).

TABLE 1

Univariate analysis, using conditional logistic regression, of the association, in multibacillary patients, between reactions after released from treatment from multi-drug therapy and biological, clinical and related to bacterial load variables.

Variables	Reaction after released from treatment						
	yes		no				
	nº	%	nº	%	Odds ratio**	95% CI for odds ratio	P
Sex							
female	28	26.9	43	41.4	1*	-	
male	76	73.1	61	58.6	2.07	(1.09-3.92)	0.025
Age							
0-35 years	48	47.1	36	34.6	1*	-	
< 35 - 59	46	45.1	48	46.1	0.65	(0.35 - 1.22)	0.190
≥ 60 years	8	7.8	20	19.2	0.26	(0.09 - 0.71)	0.009
Clinical form							
borderline	58	56.3	86	82.7	1*	-	
lepromatous	45	43.7	17	16.3	3.7	(1.84 – 7.44)	0.00
Reaction during MDT							
no	24	23.5	54	54.0	1*	-	
yes	78	76,5	46	46.0	4.33	(2.09-2.09)	0.000
Type of reaction							
neuritis	14	18.0	14	30.4	1*	-	
ENL	44	56.4	8	17.4	4.34	(1.12-16.87)	0.034
reversal reaction	20	25.6	24	52.2	0.45	(0.10-1.87)	0.275
Bacterial index at diagnosis							
negative	5	6.0	34	39.1	1*		
0.1 – 2.0	32	38.5	39	44.8	7.7	(1.74 – 34.34)	0.007
>2,0	46	55.5	14	16.1	21.9	(4.67-102.93)	0.000
Bacilloscopy							
negative	52	50.0	87	87.8	1*	-	
positive	52	50.0	12	12.2	7.21	(3.06-16.96)	0.000
ML FLOW							
negative	22	21.15	69	66.35	1*	-	
positive	82	78.85	35	33.65	10.4	(4.15-26.03)	0.000

*level of reference, **odds ratio matched for year of released from treatment from multi-drug therapy/multibacillary (±6 months)

CI: confidence intervals, ENL: erythema nodosum leprosum, MDT: multi-drug therapy.

In the multivariate analysis, the variables grouped in blocks adjusted for each other and for the ML Flow result were evaluated. The results indicated that the magnitude of the estimated odds ratio presented only slight changes, suggesting a minimal confounding effect, if at all, between the variables (**Table 2**).

TABLE 2

Multivariate analysis by blocks of variables (using the conditional logistic regression model), of the association, in multibacillary patients, between reactions after released from treatment from multi-drug Therapy and biological, clinical and related to bacterial load variables adjusted for ML flow.

Variables of the model	Odds ratio**	CI_{OR}(95 %)	P
Biological variables			
ML Flow			
negative	1*	-	-
positive	11.49	4.34 - 31.25	0.000
Age			
0-35 years	1*	-	-
> 35 – 60	1.44	0.65 - 3.22	0.359
> 60 years	0.20	0.06 - 0.68	0.011
Clinical variables			
ML Flow			
negative	1*	-	-
positive	12.99	3.84 - 40.63	0.000
Reaction (during MDT)			
no	1*	-	-
yes	8.21	2.64 - 25.48	0.000
Clinical form			
borderline	1*	-	-
lepromatous	2.94	1.01 - 8.50	0.046
Related to Bacterial Load			
ML Flow			
negative	1*	-	-
positive	5.55	1.75 - 20.00	0.004
Bacilloscopy (study)			
negative	1*	-	-
positive	2.94	0.88 - 10.00	0.080
IBI			
negative	1*	-	-
0.1 – 2.0	7.14	1.20 - 42.39	0.031
> 2.0	16.89	2.43 - 116.98	0.004

MDT: multi-drug Therapy, IBI: initial bacterial index, CI: confidence intervals.

*Level of reference, **OR paired for year of released from treatment from multi-drug therapy/multibacillary (±6 months).

DISCUSSION

The results obtained in this study indicate that a statistically significant association occurs between post-MDT treatment reaction and bacterial load, evaluated by positive serology (anti PGL-I / ML Flow) (OR: 10.4) and bacilloscopy (OR; 7.21), both examined after RFT and with an IBI > 2.0 registered before the onset of MDT.

An IBI > 2 may be a laboratorial marker of posttreatment reactions. Similar results were observed in other studies which demonstrated that this index is a risk factor for posttreatment reactions[9 15]. In the Jamet and Baohong study[9], patients that presented an IBI ≥ 4 and a BI ≥ 3 upon completion of MDT had a greater chance of relapsing and the authors suggested that monitoring these patients is essential, given that only a small number of patients present such conditions. These cases should have their laboratory work performed at referral centers with high-quality examinations.

The observation of a reduced bacterial load with continued positive bacilloscopy at RFT involving the standardized WHO treatment regimens has been observed in clinical trials in several parts of the world, including Brazil[3 9 12]. The WHO recommendations include yearly monitoring of patients that have completed MDT/WHO and the performance of bacilloscopy at RFT, based on the assertion that the risk of relapse is very low after multi-drug therapy for leprosy[12].

However, retrospective studies in the first cohorts of MB patients that received MDT/WHO in 24 fixed doses in Brazil, were conducted to evaluate the anti-PGL-I antibody using ELISA in 165 patients after an average of 50 months post-RFT. The results demonstrated a high percentage of positivity for anti-PGL-1 and a statistically significant association with BI positivity. The majority of patients presented the lepromatous form, suggesting that a group at high risk of relapse exists characterized by the following factors: lepromatous clinical form, BI positive and anti-PGL-I with optical density of 0.2 to 0.8[14]. These characteristics were also indicated in the present study as key risk factors for posttreatment reactions, indicating that posttreatment reactional episodes share common risk factors with relapse cases.

A study conducted by Gallo *et al*[7], in which MB patients took two courses of MDT, showed that a gradual reduction occurs in the mean BI in cases evaluated both during and after treatment and that reactions were diagnosed in 43% of patients after RFT. ENL was the most frequent and recurring type of reaction and a gradual decline in reactions was observed as patient follow-up increased. However, two relapse cases were diagnosed and confirmed via inoculation and in both cases ENL was reported to have continued for more than 3 years.

Based on the results of these two studies, it could be concluded that the bacilli are able to remain relatively protected from immune cellular activity and MDT. In more propitious conditions, the bacilli are subsequently able to proliferate, stimulating inflammatory reactions and causing relapses or reactions, or even reactions associated with relapses in the posttreatment period. The most important factor in the definition of these situations is the immune system of the individual in question.

Whichever treatment regimen is used, as is the case with tuberculosis, the possibility of survival and viability of so-called persister bacilli always exists, even after a negative bacilloscopy is observed in skin smears or cutaneous biopsies[10].

Shetty *et al*[18] studied borderline tuberculoid (BT) patients after completing treatment and, by means of anatomopathological exams, verified that 58.3% of patients with histopathological conditions compatible with reactions presented viable bacilli when inoculated in mouse paw (Shepard technique[17]). These findings indicate more extensive follow-up is required for patients that

suffer from post-RFT reactions, with discussion of the possibility that relapses are accompanied by reaction phenomena.

The presence of positive testing for antibodies after treatment should be monitored, principally in patients with post-RFT reactions, given the fact that this may reflect a current infection and a risk factor for future development of relapse, as observed by Wu *et al*[22], in which the risk of relapse in MB patients was 6.7-fold greater compared with cured PB patients and that the cumulative risk of relapse among patients with positive serology was 13.7% compared with 0.4% for those with negative serology.

Based on the results obtained in this study, it is important to review the WHO recommendations, as well as those of the MoH, principally in reference centers for management of posttreatment reactions, due to the fact that a bacterial load after RFT determined by bacilloscopy or serology could be an indicator of the possibility of multiplying persister bacilli, resulting in reactions or relapse.

The data presented suggest that an association exists between bacterial load (assessed by anti-PGL-I) and leprosy reactions after MDT completion, backed by multivariate analysis that shows that this association is independent of age, clinical form and the presence of reactions during treatment.

Analyzing the variables that were associated with posttreatment reactions, among which are the lepromatous clinical form, a IBI > 2, testing positive for anti-PGL-I antibodies and a positive BI after RFT, all of these are also related to relapse[8 14 22]. To a certain degree, this indicates that posttreatment reactions may be a risk factor for relapse, as theorized by Brito[3] in 2004, or that these two situations simply present common risk factors.

The ML Flow serological test to detect the anti-PGL-I antibody is a fast and simple tool that permits improved classification of HD patients that can help to determine the most adequate type of MDT, avoid incorrect classification and/or the possibility that treatment failure might be confused with relapse and identify new foci of infection. The leprosy control program should examine the possibility of using this method in patients that are at risk of developing post-RFT reactions with a view to supporting new strategies for prevention and control of the endemic.

CONCLUSION

The results of this study indicate several clinical markers for post-RFT reactions, such as: individuals aged over 60 years-old, presenting the lepromatous form of the disease and the occurrence of a leprosy reaction, principally ENL, while undergoing MDT treatment. As for laboratorial markers, these include the presence of a high bacterial load, as indicated by seropositivity or by a positive skin smear after RFT, and an IBI > 2. It essential that local health professionals are adequately prepared to handle these episodes and must be aware of these possibilities even after MDT has been fully administered.

The use of anti-PGL-I serology with ML Flow or skin smear bacilloscopy in patients with posttreatment reactions will make it possible to identify target groups for new treatment, prevention and monitoring strategies.

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