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A Study on a Possibility of Predicting Early Relapse in Leprosy Using a ND-O-BSA Based ELISA[1]

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Relapse of leprosy is a very important clinical manifestation ([7]), because relapses may occur either after monotherapy or multidrug treatment (MDT) causing it to affect the final events of prevention and treatment of leprosy. With the wide coverage of leprosy patients under MDT, more and more patients are being released from treatment after completing a relatively brief period of time on chemotherapy. In a few years, monitoring these cases for relapse will likely become an important and major task for the leprosy program. A clinical examination supported with a simple test would be very helpful in defining and diagnosing cases of relapse at a very early stage. Therefore, an immunoserological tool could be useful. In view of the fact that PGL-I based ELISA is sensitive and specific in detecting IgM antibody against PGL-I from *M. leprae* in sera of leprosy patients, and ND-O-BSA- (an immunodominant disaccharide epitope of PGL-I linked to bovine serum albumin (BSA) via an octyl linker arm) based ELISA (ND-ELISA) has been established in our laboratory, we conducted a study on the possibility of predicting relapse among leprosy patients who had completed chemotherapy. In this article, we will mainly report the results evaluated by the ND-ELISA according to the criteria for screening the disease, then following the IgM antibody levels (IgM-AbL) against ND in sera from those individuals cured after dapsone (DDS) monotherapy (post-DDS) for a period of three years. DDS was given in 100 mg to 200 mg daily doses for multibacillary patients and in 50 mg to 100 mg daily doses for paucibacillary cases. The patients were considered cured when there was no evidence of disease activity clinically in the skin or nerves; skin smears were negative for acid-fast bacilli; and skin or nerve biopsies showed no histologic changes of leprosy.

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MATERIALS AND METHODS

Blood samples. Blood samples were taken from 103 leprosy patients (classified according to the Ridley-Jopling classification as LL = 25, BL = 25, BB = 3, BT = 26 and TT = 24; 100 active tuberculosis patients whose sera were stored in our laboratory sera bank; 192 healthy individuals from a nonendemic area (92 were from the Institute of Dermatology of Prevention and Treatment for Endemic Disease, Gansu, China; 100 were from the Institute of Dermatology for Prevention and Treatment, Dalian, China), and 666 individuals cured after DDS monotherapy (354 from Gansu; 312 from Dalian). All the above blood samples were kept frozen at –20°.

Antigen. ND-O-BSA antigen (ND) was provided by Dr. Patrick Brennan (Colorado State University, Fort Collins, Colorado, U.S.A.). It was applied to 40-well, flat-bottomed polystyrene microtiter plates (Third Factory for Plastic Products, Shanghai, China). Each vial of ND contained 50 μg of sugar (as glucose equivalent) and 200 μg of BSA, and represented 0.5 ml of lyophilized material. After adding 0.5 ml of distilled water for reconstitution, this solution was diluted to a concentration of 0.1 μg/ml with a volatile buffer (0.01 M ammonium acetate-carbonate, pH 8.2) for coating the plates ([3]).

Blocking agent. Skim milk (SM), an instant nonfat dry milk (Lucerne, Honolulu, Hawaii, U.S.A.) was dissolved with phosphate-buffered saline (PBS) (Na_2HPO_4 12.8 g, NaH_2PO_4 2.62 gm NaCl 0.58 g, distilled water 1000 ml, pH 7.4). Its working concentration as 2.5% (w/v) (for diluting sera tested and conjugate) and 5% (w/v) (for blocking the antigen-coated wells).

Conjugate and color reagent. Horseradish peroxidase (HRP)-conjugated anti-human IgM (DAKO Accurate Chemicals, Westbury, New York, U.S.A.) was diluted with PBS containing 2.5% skim milk. Its working concentration was 1:1000. *O*-Phenylenediamine (OPD) (Sigma Chemical Company, St. Louis, Missouri, U.S.A.) was dissolved in citrate buffer (citric acid 4.67 g, Na_2HPO_4 7.3 g, distilled water 1000 ml, pH 5.0) and diluted to the required concentration (0.04% w/v).

ELISA. The enzyme-linked immunosorbent assay (ELISA) was essentially conducted with Dr. Brennan's procedures ([8]).

TABLE 1. *Rates of positivity in sera from various group tested in ND-ELISA.*

Group of sera tested	No. of case	No. (rate) of positivity
LL	25	25 (100.0)
BL	25	25 (100.0)
BB	3	3 (100.0)
BT	26	24 (92.4)
TT	24	22 (91.6)
Total	103	99 (96.1)
TB	100	0 (0)
NC	100*	0 (0)
	92**	4 (4.3)

Normal value (NV) (i.e., cut off value), its absorbance value is 0.16 in our study.

* Sera from Dalian.

** Sera from Gansu.

Statistical analysis. The upper limit for normal values was calculated as the mean plus two or three standard deviations of the values of the normal sera and named normal value (NV). For evaluating the significance of ND-ELISA, different parameters were used, i.e., sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), Youden's Index (YI), false-positive rate (FPR), false-negative rate (FNR), negative likelihood ratio (LR–) and positive likelihood ratio (LR+), and rank correlation (Spearman's method). In order to analyze the correlation between the IgM-AbL and relapse in leprosy, risk ratio (RR) and attributable risk (AR) were also used. The formula is: RR = Ie/Iu = a/N1/C/No and AR = Ie – Iu. RR > 1 = "positive" exposure-disease association. In the formula, Ie = incidence rate in the group exposed; Iu = incidence rate in the group unexposed; a = sum of disease in the group exposed; N1 = sum of disease and without disease in the group exposed; C = sum of disease in the group unexposed; and No = sum of disease and without disease in the group unexposed.

Criteria for relapse in leprosy. The criteria for diagnosing relapse among these treated patients was a) the appearance of new skin lesions or new activity in previously existing skin lesions or new nerve function loss or new paralysis of muscles; b) the finding of a new skin lesion or previ-

TABLE 2. *Valuation of ND-ELISA for screening infection with* M. leprae.

Indicator for estimation	Results of estimation
Sensitivity	0.960
Specificity	0.960
Positive predictive value (PPV)	0.960
Negative predictive value (NPV)	0.960
Youden's Index (YI)	0.920
False positive rate (FPR)	0.04
False negative rate (FNR)	0.04
Positive likelihood ratio (LR+)	24.00
Negative likelihood ratio (LR–)	0.041

ous lesion with a high skin smear bacterial index containing solid-staining bacilli, and c) histological evidence of relapse in a skin or nerve biopsy, where specific changes of leprosy or acid-fast bacilli were found. If two of the above criteria were met, a relapse in leprosy was diagnosed.

RESULTS

The results of the studies are shown in Tables 1 to 8. Table 1 shows the results in sera from various groups tested in ND-ELISA: a) The positivity rates of various groups *not under treatment* are LL 100%, BL 100%, BB 100%, BT 92.4%, TT 91.6%, TB 0%, normal controls 0% (in Dalian) and 4.3% (in Gansu). b) From TT to LL, the positivity rate gradually increased, and from BB to LL, there was the same percent positivity (100%), but from TT to BT, the positivity rate is different (higher in BT than in TT). c) In normal controls, the positivity rate is higher in Gasu than in Dalian. The reason will be discussed further.

Table 2 shows the results of evaluation of the ND-ELISA according to the criteria of screening for the disease. The results indicated that the sensitivity, specificity, PPV, NPV and YI were all more than 90%; the FPR and FNR were all very small and LR+ is much bigger than LR–.

Table 3 shows the relationship between BI and mean absorbance value (M_{OD}) in the ND-ELISA. The Spearman's Rank Correlation Coefficient is 6.857, indicated that there was a highly significant correlation between BI and M_{OD}.

Table 4 shows that in multibacillary (MB) and paucibacillary (PB) patients who had been cured with DDS monotherapy and who were followed uneventfully for various periods of time, the mean antibody level remained low for many years afterwards.

Table 5 shows the three-year follow-up in leprosy patients cured with DDS monotherapy: a) Among multibacillary patients, 95 were Ab positive (Ab+), and 12 of them were diagnosed as a relapse in leprosy, an additional 335 cases were Ab negative (Ab–), and only one of them was diagnosed as a relapse in leprosy. b) In paucibacillary patients, 44 were Ab+ and 192 were Ab–. There was one case of a relapse of leprosy in each group. Among all 666 cases followed after cure with DDS monotherapy, a total of 15 cases of a relapse in leprosy was found. c) The risk of relapse was 6.7 times higher in multibacillary than in paucibacillary patients followed for three years after being considered cured with DDS monotherapy.

As shown in Table 6, in the Ab+ group, the cumulative rate of relapse (CRR) was 13.68%, and in the Ab– group, the CRR was 0.35%, for a risk ratio (RR) = 36.7. From Table 7, we should observe that, at

TABLE 3. *Correlation between BI and M_{OD} in ND-ELISA (Spearman's method).*

Group	No. of cases	BI(+) (x)	M_{OD} (y)	Rank for		Difference of rank (d)–	d^2
				BI	M_{OD}		
I	15	0	0.24	1	1	0	0
II	2	1	2.09	2	4	–2	4
III	5	2	1.29	3	2	–1	1
IV	16	3	1.73	4	3	1	1
V	13	4	2.18	5	5	0	0
VI	9	5	2.64	6	7	–1	1
VII	1	6	2.27	7	6	1	1
Total	561						$8(\Sigma d^2)$

rs(ND) = 6.857>0.786; $p < 0.05$; M_{OD} = the mean absorbance value at 490 nm.

TABLE 4. *Relevance of IgM-antibody level in sera from leprosy patients cured with DDS monotherapy (post-DDS) to a period of time after curing.*

Period of time after curing	MB of post-DDS[b]		PB of post-DDS[c]	
	No. of cases	M_{OD}[a]	No. of cases	M_{OD}
0–5	13	0.138 ± 0.036	1	0.080 ± 0
6–10	39	0.098 ± 0.040	11	0.100 ± 0.049
11–15	64	0.093 ± 0.098	22	0.078 ± 0.071
16–20	51	0.092 ± 0.052	13	0.079 ± 0.039
21–25	72	0.078 ± 0.055	20	0.085 ± 0.053
>25	31	0.078 ± 0.061	17	0.074 ± 0.052
Total	270		84	

[a] M_{OD} = mean optical density. It represents the level of IgM antibody (IgM-AbL) from leprosy patients.
[b] MB of post-DDS = multibacillary leprosy cured after DDS monotherapy.
[c] PB of post-DDS = paucibacillary leprosy cured after DDS monotherapy.

the time of relapse, the antibody levels were all positive except one case of TT; and the antibody levels gradually decreased in all the relapsed leprosy cases after they were put back on effective treatment. The period of time for a relapse was 14–34 years after being cured, with no clustered period found, and changes in type (TT → BT) might develop, although it was rare. Table 8 shows that, even though *some of* the samples were from paucibacillary patients being followed after cure with DDS, the antibody levels were positive at the time of relapse in the majority of them. Usually the relapse did not develop until the consistent positivity of antibody levels or gradually increasing values. Interestingly, with the appearance of a relapse within one to two years (two years in the majority of cases) after antibody levels became positive, the period of time for a relapse was 22–33 years, with no clustered period and no change of leprosy type.

DISCUSSION

In this article, we wanted to determine whether the ND-ELISA could be useful in detecting and monitoring relapse in leprosy or not. First, we evaluated the value of ND-ELISA for screening infection with *M. leprae.* We then measured antibody levels in patients who had been cured with DDS monotherapy to understand how long the levels of IgM-antibody remain positive after cure. From these data we were able to establish the reference baseline of levels of antibody for detecting relapse in leprosy. Correlations between the antibody levels and the clinical diagnosis of relapse allowed us to confirm the practical usefulness of measuring antibody levels in predicting relapse before the clinical signs developed. According to our results, some pertinent discussions follow.

Evaluation of ND-ELISA for screening infection with *M. leprae.* The results of ND-ELISA in Table 2 (derived from Table 1) indicates that sensitivity, PPV and NPC are all equal to 0.96, YI is 0.92; FPR and FNR are all equal to 0.04, meanwhile LR+ is 24, which is greater than LR– (0.041). These data suggest the ND-ELISA is an excellent method of screening for infection with *M. leprae.* Table 1 shows that the

TABLE 5. *Three-year follow-up results of relapse in leprosy cured post-DDS in ND-ELISA.*

Source of sample	No. of case	Antibody status		No. of relapse	
		No. of Ab+	No. of Ab–	Ab+	Ab–
MB of post-DDS	430	95	335	12(2.8)*	1
PB of post-DDS	236	44	192	1(0.42)*	1
Total	666	139	527	13	2

RR = risk ratio = 6.7; Ab+ = IgM antibody positive; Ab– = IgM antibody negative.
* The number in parentheses = percentage (%).

TABLE 6. *Cumulative rate of relapse in leprosy in ND-ELISA during three-year follow-up.*

Ab status	No. of relapse	No. of without relapse	Total	Rate of relapse (%)
Ab+	13	82	95	13.68
Ab–	2	569	571	0.35
Total	15	651	666	2.25

RR = 36.7; AR = attributable risk = 13.33%.

mean antibody levels gradually decreased from LL to TT. This suggests the possibility of correlation between the spectrum of leprosy and levels of IgM antibodies in patients. Table 3 shows that there is a highly significant correlation between bacterial index and antibody levels. Thus, changes in antibody levels predict changes in bacterial index or total body load of leprosy bacilli. This has been applied in detecting early infections with *M. leprae.*

Further, if one wants to detect the relapse in leprosy, it is very important to understand the behavior of antibody levels after patients are cured with DDS monotherapy and DDS is discontinued. The results shown in Table 4 indicate that the antibody levels in both multibacillary and paucibacillary patients gradually decreased after effective treatment, and again suggest that there is a correlation between antibody levels and bacillary load. This is the basis for predicting relapse in leprosy at an early state by measuring antibody levels.

Association of IgM-AbL with relapse in leprosy. As shown in Table 5, a) the numbers of relapses in leprosy were more pronounced in antibody positive individuals than in antibody negative individuals. b) The risk of relapse was higher in multibacillary patients than in paucibacillary patients. These results are identical to those published by Chin-A-Lien, *et al.* ([1]) and Buhrer-SeKula, *et al.* ([5]). c) Although the numbers of relapses in antibody negative individuals were much lower, relapses were still to be found. This result is identical to that reported by Buhrer-SeKula, *et al.* ([5]). The results in Table 5 and Table 6 suggest that ND-ELISA positivity is a risk factor for the future development of relapse, and that this test is useful in detecting relapses in leprosy, especially relapses in multibacillary leprosy.

Furthermore, the results shown in Table 7 and Table 8 indicate the association of relapse in leprosy with antibody positivity. At the time of relapse, antibody levels were positive in the majority of individuals cured with dapsone monotherapy. Relapses did not develop until one or two years after antibody levels became consistently positive.

These data indicate that the ND-ELISA is a useful tool for predicting and detecting the early relapse of leprosy. Interestingly, among these patients cured with DDS monotherapy, the time for relapse was 12 years to 33 years after DDS was discontinued with no clustered period, and with a change of leprosy classification occurring only rarely.

In our studies, the sensitivity and specificity were higher in the ND-ELISA than in those in the dipstick assay, other ND- or PGL-I-ELISA. We think the main reasons may be that the principle and procedures of the dipstick assay are different from the ND-ELISA we are using. As for other ND-

TABLE 7. *Changes of levels of IgM antibody in individuals cured post-DDS who were diagnosed as relapse of leprosy revealed at the first detecting after effective treatment.*

Individual detected	OD (at relapse)	Change of Ab level				Incubation of relapse (yr)	Change of type
		1 yr	2 yr	3 yr	FT		
022 (♂)	0.275	0.275	0.190	0.150	↓	15	TT → BT
037 (♂)	0.285	0.285	0.205	0.170	↓	25	LL → LL
070 (♀)	0.270	0.270	0.185	0.155	↓	16	LL → LL
133 (♂)	0.660	0.660	0.350	0.170	↓	14	LL → LL
167 (♂)	0.680	0.680	0.160	0.160	↓	22	LL → LL
107 (♂)	0.035	0.035	0.080	0.010	?	26	TT → TT
100*(♂)	0.305	0.305	0.130	D	↓	34	LL → LL

* PCR = positive; ♂ = Male; ♀ = Female; D = died; yr = year; ↑ = increase; ↓ = decrease; Ab = IgM antibody; OD = optical density; FT = final trend.

TABLE 8. *Status of level of IgM-antibody in individuals cured post DDS who were diagnosed as relapse of leprosy during continuing three-year follow-up.*

Individual detected	OD (at relapse)	Trend of monitoring Ab				Time of relapse after sero-conversion (yr)	Incubation of relapse (yr)	Change of type
		1 yr	2 yr	3 yr	FT			
039 (♂)	0.405	0.040	0.500	0.405	↑	1	27	LL → LL
058 (♀)	0.260	0.250	0.260	0.260	=[a]	2	15	LL → LL
073 (♂)	0.205	0.065	0.185	0.205	↑	1	27	LL → LL
128 (♂)	0.290	0.175	0.260	0.290	↑	2	12	LL → LL
339 (♀)	0.265	0.115	0.145	0.265	↑	2	33	LL → LL
340 (♀)	0.715	0.190	0.350	0.715	↑	2	26	TT → TT
344 (♀)	0.450	0.170	0.285	0.450	↑	2	16	LL → LL
021 (♂)	0.030	0.110	0.030	0.030	↓	2	21	LL → LL

[a] Represents level of IgM antibody stability.

or PGL-I-ELISA in the majority of tests reported, BSA was usually used as a blocking agent. Choosing the optimum concentration of BSA for blocking non-specific binding in the reaction is very important. As is well-known, blocking efficiency effects the cut-off value in the ELISA, as well as the sensitivity and specificity. Again, under optimum concentration, the blocking efficiency is higher in skimmed milk than it is in BSA according to our experience ([4]). Additionally, in this study the positivity rate (4.3%) is higher in the normal controls from Gansu than those (0%) in the normal controls from Dalian. This suggests that the normal values are influenced by the prevalence of leprosy in the area selected.

CONCLUSION

We consider the ND-ELISA to be useful in screening for early infection with *M. leprae* and that it has practical potential in predicting and monitoring relapse in leprosy, especially in multibacillary leprosy. This conclusion is also consistent with those reported by Cho ([2]), Chin-A-Lien ([1]) and Naafs ([6]), but the period of time for relapse was interestingly 12 years to 33 years.

SUMMARY

Serological methods have been used for detecting infection with *Mycobacterium leprae.* We have applied a serological test to explore the possibility it could detect a bacterial relapse among patients who have been cured with chemotherapy. More specifically we used an indirect enzyme-linked immunosorbant assay (ELISA) using the natural disaccharide (ND) of the phenolic glycolipid antigen of *M. leprae* linked to bovine serum albumin as antigen. Antibody levels were measured in sera from normal controls, active leprosy cases, cured leprosy patients, and relapsing leprosy patients. We correlated antibody levels with the type of leprosy, the bacterial index, and with relapse among cured leprosy patients.

In our hands, the ND-ELISA, when applied to screening for infection with *M. leprae,* had excellent sensitivity, specificity, positive and negative predictive values, and both a low false positive rate and a low false negative rate. Antibody levels gradually increased among active patients from the tuberculoid to the lepromatous end of the leprosy spectrum. There was a year-by-year fall in antibody levels in patients responding to chemotherapy. Antibody levels and the bacterial index were correlated using the Spearman's rank correlation method.

Serial antibody levels were measured in 666 leprosy patients after being cured with dapsone monotherapy. Over a three year follow up, 95 multibacillary patients became antibody positive and 12 of them had bacterial relapses of their disease. In contrast, among 335 cases that remained antibody negative, only one relapse was seen. Among 44 paucibacillary cured patients who became antibody positive, there was one relapse. There were 192 such patients who remained antibody negative and one relapsed.

The risk of relapse is 6.7 times higher among cured multibacillary patients compared to cured paucibacillary patients. Overall, the cumulative relapse rate among

antibody positive cases was 13.7%, compared to 0.4% among antibody negative patients. We conclude that the ND-ELISA is a useful tool both for screening for early infection with *M. leprae* and for predicting a relapse in cured patients, particularly in cured multibacillary patients.

RESUMEN

Se han usado varios métodos serológicos para detectar la infección por *Mycobacterium leprae.* Nosotros hemos aplicado una prueba serológica para explorar la posibilidad de que con ella se puedan detectar las recaídas bacterianas entre los pacientes que han sido curados con quimioterapia. Más especificamente, hemos utilizado un inmunoensayo enzimático (ELISA) usando como antígeno el disacárido natural (DN) del glicolípido fenólico de *M. leprae,* unido a albúmina sérica bovina. Los niveles de anticuerpos se midieron en el suero de controles sanos, casos activos de lepra, pacientes con lepra curados, y pacientes con lepra en recaída. Se buscó la correlación entre los niveles de anticuerpo y el tipo de lepra, el índice bacteriano, y la tasa de recaída entre los pacientes con lepra curada.

En nuestras manos, el ELISA-DN, aplicado para buscar la infección con *M. leprae,* tuvo excelentes grados de sensibilidad, especificidad, valores predictivos positivos y negativos, y bajos índices de resultados falsos positivos y negativos. Los niveles de anticuerpos disminuyeron gradualmente del extremo lepromatoso al extremo tuberculoide en los casos activos. En los pacientes que respondieron a la quimioterapia, los niveles de anticuerpos mostraron una caída anual sostenida. Los niveles de anticuerpos y el índice bacteriano se analizaron usando el método de correlación de rangos de Spearman.

Los niveles de anticuerpos se midieron periódicamente en 666 pacientes con lepra que habían sido curados con monoterapia con dapsona. En un periodo de seguimiento de 3 años, 95 pacientes multibacilares llegaron a ser anticuerpo-positivos y 12 de ellos tuvieron recaídas baciloscópicas de su enfermedad. En contraste, entre 335 casos que permanecieron anticuerpo-negativos, solo se vio un caso de recaida. Entre los 44 pacientes paucibacilares curados que llegaron a ser anticuerpo-positivos, hubo un caso de recaída. Hubieron 192 pacientes que permanecieron anticuerpo-negativos y uno con recaída.

El riesgo de recaída es 6.7 veces más alto entre los pacientes multibacilares curados que entre los pacientes paucibacilares. Globalmente, la tasa cumulativa de recaída entre los casos anticuerpo-positivos fue del 13.7%, comparado con 0.4% entre los pacientes anticuerpo-negativos. Concluimos que el ELISA-DN es una herramienta útil tanto para la búsqueda de la infección temprana con *M. leprae* como para la predicción de recaídas en los pacientes curados, particularmente en los pacientes multibacilares curados.

RÉSUMÉ

Des méthodes sérologiques ont déjà été utilisées pour détecter une infection par *Mycobacterium leprae.* Nous avons appliqué une méthode sérologique afin de tester l'hypothèse qu'elle pourrait permettre de détecter une rechute bactérienne parmi les patients ayant été auparavant traités avec succès par la polychimiothérapie. Plus précisément, nous avons mis en œuvre une méthode immuno-enzymatique indirecte de type ELISA utilisant comme antigène le disaccharide naturel (ND) de l'antigène glycolipidique phénolique de *Mycobacterium leprae* couplé à l'albumine sérique bovine. Les taux d'anticorps furent mesurés dans le sérum de témoins normaux, de cas de lèpre active, de patients lépreux guéris et de patients hanséniens en rechute. Une corrélation fut effectuée entre les taux en anticorps d'une part, et le type de lèpre, l'index bactérioscopique et le taux de rechutes parmi les patients lépreux guéris d'autre part.

Entre nos mains, la méthode ND-ELISA, lorsqu'appliquée au dépistage de l'infection par *Mycobacterium leprae,* présenta une excellente sensibilité, spécificité, d'excellentes valeurs prédictives positives et négatives ainsi que de faibles taux de faux-positifs et de faux-négatifs. Les niveaux d'anticorps augmentèrent progressivement parmi les patients en lèpre active du pôle tuberculoïde au pôle lépromateux du spectre des manifestations clinico-pathologiques de la lèpre. Il y eut une diminution année par année des taux d'anticorps chez les patients répondant à la polychimiothérapie. Les taux d'anticorps et l'index bactérioscopique furent corrélés en utilisant la méthode de corrélation de variables discrètes selon Spearman.

Des mesures sériées d'anticorps furent réalisées chez 666 patients lépreux guéris par la monothérapie à la Dapsone. Pendant un suivi d'une durée de trois ans, 95 patients multibacillaires devinrent positifs et 12 d'entre eux montrèrent une rechute bactérienne de leur maladie. En revanche, parmi 335 cas qui restèrent négatifs au test ND-ELISA, seule une rechute fut détectée. Parmi 44 patients paucibacillaires guéris qui devinrent positifs à la détection des anticorps, il y eut une rechute. Il y eut 192 patients paucibacillaires qui restèrent négatifs au test sérologique et une rechute fut observée.

Le risque de rechute fut 6,7 fois plus élevé parmi les patients multibacillaires que parmi les patients paucibacillaires. Globalement, le taux cumulé de rechutes parmi les cas positifs aux anticorps était de 13,7% tandis qu'il était de 0,4% chez les patients négatif aux anticorps. Nous concluons que le ND-ELISA est un outil tout à fait utile à la fois pour le dépistage de l'infection précoce avec *Mycobacterium leprae* et pour prédire une rechute chez les patients guéris, en particulier ceux ayant souffert de lèpre multibacillaire.

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