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SEROEPIDEMIOLOGICAL SURVEYS FOR LEPROSY IN ECUADOR

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Abstract: Serological examination of leprosy in endemic areas of cutaneous leishmaniasis were carried out using the sera collected during a survey for cutaneous leishmaniasis and several parasitic diseases in Ecuador. There was no correlation between prevalence rates for leprosy and seropositive rates of the antibodies (anti-PGL-I and LAM-B antibodies) in the subjects living in several provinces in Ecuador. Seropositive rates of anti-PGL-I antibodies of the leprosy patients and their families in Los Ranchos, Department of Manabi, were relatively high (84.6%, 11/13) in comparison with the average seropositive rates (42.4%, 154/365) of the subjects from other areas of Ecuador. It was suggested that serological survey of families of leprosy patients might be useful for screening of household contacts in a low endemic areas, such as Department of Manabi, Ecuador.

INTRODUCTION

Leishmaniasis and leprosy are etiologically completely different diseases, but it has been known that the two diseases cause immunologically similar responses in their hosts (Bryceson, 1981). Therefore, it may be important to know seroepidemiological features of leprosy in endemic areas of cutaneous leishmaniasis. Two types of skin tests, Lepromin test for leprosy and Leishmania (Montenegro) skin test for leishmaniasis, were made on several leprosy patients and thier families. Using sera which were collected during surveys for cuteneous leishmaniasis and other parasitic diseases inculuding leprosy, the value of anti-PGL-I (phenolic glycolipid-I) antibodies and anti-LAM-B (Lipoarabinomannan-B) antibodies were measured for the serological studies of leprosy. PGL-I is a major secretory product of *Mycobacterium leprae* (Hunter, *et al.*, 1982). LAM-B is a complex glycolipid found in large amounts (15 mg per g of bacilli) within the cell walls of *M. leprae* and *M. tuberculosis* (Gaylord and Brennan, 1987). The present paper reports the result of preliminary serological examinations of leprosy. Furthermore, based on the results obtained, a brief comment was also made on the screening method to detect leprosy patients in early stage of the disease.

MATERIALS AND METHOD

Subjects examined

In this preliminary epidemiological survey of leprosy, an evaluation was made on the serodiagnosis of the subjects from the following areas of Ecuador: Los Ranchos, Portoviejo and Guayabales, Department of Manabi; Echeandia, Department of Bolivar; Antepara, Machala, Pinas, Portovelo and Zaruma, Department of El Oro; Pedro Carbo, Guayaquil and Olon, Department of Guayas; Selva Alegre, Department of Esmeraldas; and other areas of Ecuador. A total of 365 subjects (153 males, 184 females and 28 unknown), with 3 to 73 years

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old, were examined for anti-PGL-I antibodies (IgG and IgM) and LAM-B antibodies (IgG and IgM). The mean age of subjects was 26.2-year-old in male, 25.4-year-old in female and 25.8-year old in total. In the present subjects from different areas, the following underlying diseases were reported: Department of Bolivar, cutaneous leishmaniasis, 15; El oro, Chagas' disease, 15 and gnathostomiasis, 1; Guayas, cutaneous leishmaniasis, 13, Chagas' disease, 9, gnathostomiasis, 6, toxoplasmosis, 4; Pichincha, cuteneous leishmaniasis, 17, Chagas' disease, 1; Esmeraldas, cuteneous leishmaniasis, 25.

Sera

Serum samples from 365 subjects were examined. These sera were collected during the surveys for several infectious diseases, such as cutaneous leishmaniasis, gnathostomiasis and Chagas' disease, including leprosy.

Skin test

The lepromin tests were performed in eight subjects suspected for leprosy. An amount (0.1 ml) of Mitsuda lepromin solution was injected intradermally on the flexor surface of the forearm using the small needle (a disposable needle; size 26G), and the skin test area was observed for erythema and induration (induration/erythema in mm) at 48 hours later. Erythema size of more than 11 mm in diameter at the injection site was considered positive reaction, and reaction with 7 × 10 mm was considered an undetermined (+/−) Mitsuda early reaction.

Leishmania skin tests (Furuya, *et al.*, 1989) were made in 13 subjects suspected of leprosy (file number G-1 - G-13). An amount (0.1 ml) of *Leishmania* promastigotes antigen solution was injected intradermally on the flexor surface of the forearm. The skin test area was observed for erythema and induration (induration/erythema in mm) at 48 hours later. Induration size of more than 5 mm in diameter at the injection site was considered as a positive reaction.

Serological examination

Blood samples were collected by venipuncture. The sera was separated with a centrifuge at several field laboratories in Ecuador. The sera were stocked in a freezer at the temperature of −20 °C. The value of anti-PGL-I antibodies and anti-LAM-B antibodies were measured by enzyme-linked immunosorbent assey (ELISA) in a laboratory at the National Institute for Leprosy Research in Japan (Jzumi *et al.*, 1993). Cut-off levels are as follows: PGL-I-IgG 0.08 OD (optical density) units; PGL-I-IgM 0.38 OD units; LAM-B-IgG 0.25 OD units; LAM-B-IgM 0.05 OD units. A criterion for considering the diagnosis was made as follows: PGL(+) and LAM(−), suspected leprosy; PGL(−) and LAM(+), suspected acid-fast bacteria infection including leprosy; PGL-IgG(+) and IgM(−), suspected leprosy (old leprosy and spontaneousry healing subjects); PGL-IgG(−) and IgM(+), suspected leprosy.

The diagnosis of leprosy was made on clinical, bacteriological and immunological grounds, according to the Rjdley-Jopling classification (Ridley and Jopling, 1996) by the doctors from the Welfare Ministry of Ecuador.

RESULTS

The results obtained are summarized as shown in Tables 1 to 6. There was no correlation between prevalence rates for leprosy and seropositive rates of the

Table 1 Prevalence rates of leprosy and anti-PGL-I* antibody positive subjects from different areas of Ecuador.

Department**	Prevalence rate (×1000)	No. examined	Positive for PGL-I Total (%)	Male	Female
1. Bolivar	0.63—0.91	15	3 (20.0%)	1	2
2. El oro	0.63—0.91	16	3 (18.7%)	3	0
3. Guayas	0.27—0.38	198	61 (30.8%)	16***	32***
4. Pichincha	0.10—0.16	18	10 (55.6%)	5	5
5. Manabi	0.10—0.16	15	12 (80.0%)	5	6
6. Esmeraldas	0.10—0.16	98	64 (61.2%)	23	41
7. Other areas		5	1 (20.0%)	0	1
Total		365	154 (42.2%)	53 (37.9%)	87 (62.1%)

*, PGL-I: phenolic glycolipid-I; **, 1. Bolivar, Echeandia; 2. El Oro, five resions; 3. Guayas, Pedro Carbo and Guayaquil; 4. Pichincha, Puerto Quito and Quito; 5. Manabi, Los Ranchos; 6. Esmeraldas, Selva Alegre; ***, Sex of 13 subjects were unknown.

Table 2 Correlation between anti-PGL-I* (IgG) and anti-PGL-I(IgM) antibodies of subjects from differnt areas of Ecuador.

Departments and cities for villages	No. and (%) of subjects in each category				Total
	PGL(IgG)+ PGL(IgM)+	PGL(IgG)+ PGL(IgM)−	PGL(IgG)− PGL(IgM)+	PGL(IgG)− PGL(IgM)−	
1. Bolivar	0 (0.0)	1 (6.7)	2 (13.3)	12 (80.0)	15
Echeandia	0	1	1	7	9
San Francisco	0	0	1	5	6
2. El Oro	0 (0.0)	2 (12.5)	1 (6.3)	13 (81.2)	16
Antepara	0	1	0	0	1
Machala	0	0	0	2	2
Piñas	0	0	1	4	5
Portovelo	0	1	0	4	5
Zaruma	0	0	0	2	2
?	0	0	0	1	1
3. Guayas	4 (2.0)	8 (4.1)	49 (24.7)	137 (69.2)	198
Pedro Carbo	3	6	41	115	165
Guayaquil	0	0	2	13	15
Olon	1	1	0	2	4
Others	0	1	6	6	13
4. Pichincha	2 (11.1)	2 (11.1)	6 (33.3)	8 (44.5)	18
Puerto Quito	2	1	6	8	17
Quito	0	1	0	0	1
5. Manabi	4 (26.7)	1 (6.6)	7 (46.7)	3 (20.0)	15
Los Ranchos	4	0	7	2	13
Portoviejo	0	0	0	1	1
Guale	0	1	0	0	1
6. Esmeraldas	12 (12.3)	11 (11.2)	41 (41.8)	34 (34.7)	98
Selva Alegre	12	11	41	34	98
7. Other areas	0	0	1	4	5
Total	22 (6.0)	25 (6.9)	107 (29.3)	211 (57.8)	365

*, PGL-I: phenolic glycolipid-I.

antibodies (anti-PGL-I- and LAM-B antibodies) in the subjects living in several provinces in Ecuador. The seropositive rates of anti-PGL-I antibodies in Ecuador was 42.2%. The seropositive rates for PGL-I was higher in female than in male. Correlation between anti-PGL-I-IgG antibodies (PGL-IgG) and anti-PGL-I-IgM antibodies (PGL-IgM) was summerized in Table 2. The distribution of anti-LAM-B antibodies in the subjects with negative anti-PGl-I antibodies in the patients with cutaneous leishmaniasis, gnathostomiasis, Chagas' disease and others was summarized in Table 3. In 211 PGL-I seronegative persons, 128 (60.7%) were found to be LAM-B seropositive; 16 (7.7%), IgG-positive and IgM-positive; 16 (7.7%), IgG-positive and IgM-negative; 96 (46.10%), IgG-negative and IgM-positive; and 80 (38.5%), IgG-negative and IgM-negative. The subjects of file number EH57 and EH119 from Esmeraldas and G-7 from Manabi showed positive reaction to all the anti-PGL-I and anti-LAM antibodies examined. The number of anti-LAM-B-IgG antibody positive nonleprous subjects in Ecuador was 69 (19.6%) out of 352.

The results of examinations of leprosy patients and their family in Los Ranchos, Department of Manabi were summarized in Tables 4 to 6. The number of subjects examined for serological tests of leprosy was 13 (7 males and 6 females). The results of skin tests were shown as follows. Only one subject, G-1, showed undetermined (+/−) Mitsuda early reaction. Seven other subjects proved to be Mitsuda negative. Leprosy patients (G-7, G-10 and G-13) except G-1 showed positive raection to the Leishmania skin test, while 10 other subjects showed negative reaction to the skin test (Table 4). A subject, G-7, showed all positive reaction against PGL-IgG and -IgM, and LAM-IgG- and -IgM. He was a borderline lepromatous leprosy patient who still had active symptoms, such as infiltrated erythema, ulcerative lesions of feet and neuralgia on the four extremities. The patient was treated with multi-drug therapy (MDT). Other two leprosy patients, G-10 and G-13, were positive for PGL-IgM, and LAM-IgG and

Table 3 Distribution of anti-LAM-B* antibodies in the subjects, negative for anti-PGL-I antibodies, from differnt areas of Ecuador.

Departments	No. and (%) of subjects in each category				Total
	LAM(IgG)+ LAM(IgM)+	LAM(IgG)+ LAM(IgM)−	LAM(IgG)− LAM(IgM)+	LAM(IgG)− LAM(IgM)−	
1. Bolivar	1 (8.3)	1 (8.3)	6 (50.0)	4 (33.4)	12
Echeandia	0	1	3	3	7
San Francisco	1	0	3	1	5
2. El Oro	1 (7.7)	2 (15.4)	4 (30.8)	6 (46.1)	13
Antepara	0	0	0	0	0
Machala	0	0	1	1	2
Piñas	1	1	0	3	5
Portovelo	0	1	3	0	4
Zaruma	0	0	0	1	1
Others	0	0	0	1	1
3. Guayas	8 (5.9)	9 (6.6)	65 (47.8)	54 (39.7)	136
Pedro Carbo	7	8	58	42	115
Guayaquil	0	1	4	8	13
Olon	0	0	2	0	2
Others	1	0	1	4	6
4. Pichincha	3 (37.5)	2 (25.0)	0 (0.0)	3 (37.5)	8
Puerto Quito	3	2	0	3	8
Quito	0	0	0	0	0
5. Manabi	0 (0.0)	0 (0.0)	1 (33.3)	2 (66.7)	3
Los Ranchos	0	0	1	1	2
Portoviejo	0	0	0	1	1
Guayabales	0	0	0	0	0
6. Esmeraldas	3 (8.8)	2 (5.9)	20 (58.8)	9 (26.5)	34
Selva Alegre	3	2	20	9	34
7. Others	0	0	0	2(100.0)	2
Total (%)	16 (7.7)	16 (7.7)	96 (46.1)	80 (38.5)	208

*, LAM-B: lipoarabinomannan-B; **, PGL-I: phenolic glycolipid-I.

-IgM. A leprosy patient, G-10, with borderline tuberculoid leprosy had relatively deep ulcers on both soles. The type of leprosy in G-13 was unknown and the subject had no specific dermatological findings of leprosy. All the leprosy patients were LAM-IgM-positive, and the subjects, G-1, G-7 and G-10 except G-13, were strongly positive for LAM-IgM, showing more than 0.190 OD units. Correlation between anti-PGL-I-IgG and anti-PGL-I-IgM antibodies in the patients with leprosy and their families was summarized in Table 5. Two leprosy patients, G-1 and G-7, were positive for PGL-IgG and -IgM. Other two leprosy patients, G-10 and G-13, were PGL-IgG-negative and PGL-IgM-positive. Correlation between anti-PGL-I antibodies and anti-LAM-B antibodies in the patients and their families was summarized in Table 6. Three patients with leprosy, G-7, G-10 and G-13, were PGL-IgM-positive and LAM-B-IgG-positive. Indeterminate leprosy patient, G-1, was negative for only LAM-IgG. There were no subjects who were PGL-IgM-negative and LAM-IgG-positive.

COMMENT

Leprosy has a wide range distribution in the world. The disease is included among the six most important infectious diseases which the World Health Organization (WHO) planned to stop from being an endemic. In some regions of Ecuador, the prevalence rates are very high. For example, the rate in Department of Los Rios showed 1.17 per 1,000 habitants. Persons living in the regions, where the prevalence rate is over 1.0 per 1,000, would be exposed to serious danger of infection whether they have or not contact with leprosy patients. The immediate counter plan for chronic infectious disease should be considered.

Recently, serodiagnosis of leprosy was considered as one of the useful methods for early diagnosis (Buchanan, *et al.*, 1983). Although, we could not examine large numbers of leprosy patients during the current

Table 4 Leishmania skin test and Lepromin test in three leprosy families in an endemic area of cutaneous leishmaniasis, Los Ranchos, Department of Manabi, Ecuador, in 1992.

No. (File no.)	Age	Sex	Lepromin test*	Leishmania skin test	Symptoms	Type of leprosy
1 (G-1)	12	F	(+/−)**	(−)	hypopigmented freckle with anesthesia	indeterminate leprosy
2 (G-2)	10	F	(−)	(−)	none	
3 (G-3)	9	F	(−)	(−)	none	
4 (G-4)	4	F	(−)	(−)	none	
5 (G-5)	11	F	(−)	(−)	none	
6 (G-6)	38	F	(−)	(−)	none	
7 (G-7)	41	M	(−)	(+)	annular and/or infiltrated erythemata, nodules with anesthesia	borderline-lepromatous
8 (G-8)	8	M	(−)	(−)	none	
9 (G-9)	15	M		(−)	none	
10 (G-10)	31	M		(+)	neuralgia, ulcers with anesthesia	borderline-tuberculoid
11 (G-11)	6	M		(−)	none	
12 (G-12)	3	M		(−)	none	
13 (G-13)	56	M		(+)	neuralgia, anesthesia	leprosy***

* Mitsuda early reaction; **, Mitsuda early reaction is undetermined with 5.7×7.5mm(+/−); *** leprosy type is unknown.

Table 5 Correlation between anti-PGL-I* (IgG) and anti-PGL-I (IgM) antibodies in the leprosy patients and their household contacts in Los Ranchos, Department of Manabi, Ecuador, in 1992.

Category	PGL (IgG) + PGL (IgM) +	PGL (IgG) − PGL (IgM) +	PGL (IgG) + PGL (IgM) −	PGL (IgG) − PGL (IgM) −
File no.	G-1, G-5 G-7, G-12	G-2, G-3, G-4 G-6, G-8, G-10 G-13	none	G-9, G-11
No. of leprosy	2	2	0	0
Total	4	7	0	2

* PGL-I: phenolic glycolipid-I;

Table 6 Correlation between anti-PGL-I* and anti-LAM-B** antibodies in leprosy patients and their household contacts in Los Ranchos, Department of Manabi, Ecuador, in 1992.

Category	PGL (IgM) + LAM (IgG) +	PGL (IgM) + LAM (IgG) −	PGL (IgG) + LAM (IgM) −	PGL (IgM) − LAM (IgM) −
File no.	G-4, G-7, G-8 G-10, G-13	G-1	G-2, G-3 G-5, G-6, G-12	G-9, G-11
No. of leprosy	3	1	0	0
Total	5	1	5	2

* PGL-I: phenolic glycolipid-I; ** LAM-B: lipoarabinomannan-B;

survey, anti-PGL-I and anti-LAM-B antibodies were examined by using accepted sera from different areas of Ecuador. The seropositive rates of anti-PGL-I antibodies in Ecuador was high, showing a rate of 42.2%. Recently, anti-LAM-B antibodies have been measured to detect the subjects with multibacillary leprosy whose values of anti-PGL-I antibodies are very low (Izumi *et al.*, 1993). In 215 PGL-I seronegative persons, 132 (61.4%) were found to be LAM-B seropositive. Some of them have the possibility of showing symptoms of lep-

rosy. Anti-PGL- and anti-LAM-B antibody positive subjects would need detailed medical examination to rule out the disease by acid-fast bacteria. A total of 154 subjects (42.2%) were serologicaly suspected of suffering from *M. leprae* and 128 subjects (35.1%) were suspected of diseases caused by acid-fast bacteria including *M. leprae*. In the examination, tuberculosis, tuberculosis cutis and its related diseases such as lupus vulgaris, tuberculosis verrucosa cutis, scrofuloderma, erythema induratum Bazin and infectious diseases of atypical mycobacteria should be taken into consideration.

Izumi *et al.*, (1993) reported the distribution of anti-LAM-B antibodies in non-leprous sera in Japan and South Sulawesi, Indonesia. According to their studies, the numbers of anti-LAM-B-IgG- antibody positive non-leprous subjects in Japan and Indonesia were 18 (4.9%) out of 367 and 20 (12.4%) out of 161, respectively. In comparison, the positive rate of anti-LAM-B-IgG antibodies in Ecuador was relatively high, showing a rate of 19.6%. From these results, we suspected that there might be large numbers of inhabitants infected subclinically by the bacillus and some of them have the possibility of showing symptoms of the disease.

In the present study, no correlation between positive rates of antibodies (anti-PGL-I and anti-LAM-B antibodies) and prevalence rates of leprosy was observed in each area of Ecuador. In Manabi, Ecuador, the prevalence rate of leprosy was 0.10-0.16 per 1,000 inhabitants, showing a relatively low rate. But the anti-PGL-I-seropositive rates of the patients and their family in Los Ranchos, Manabi (84.6%) were higher than the average positive rate (42.2%) of all subject in several areas in Ecuador. From the data shown in Table 5, it was considered that PGL -IgG and IgM- positive cases were bacteriologically and immunologically active patients.

In general, the values of anti-PGL-IgM and LAM-B-IgG antibodies were used for serological diagnosis of leprosy because the combination thought to be clinically useful for the diagnosis in early stage of the disease. As shown in Table 6, three leprosy patients were positive for PGL-IgM and LAM-IgG. The value of anti-LAM-B-IgM antibody was thought to be unreliable, because of the low of cut off value (>0.19 OD unit). But a strong positivity of LAM-IgM might be an indicator for the diagnosis of leprosy, because three out of four leprosy patients, were strongly positive for LAM-IgM (data not shown). As the subjects, G-5 and G-12, were positive for PGL-IgG and -IgM, they should be watched for the development of the disease symptoms, though no clinical findings of leprosy were observed in the present examination.

Correlation of the skin tests between cutaneous leishmaniasis and leprosy was partly found; three out of four leprosy patients showed positive reaction to *Leishmania* promastigotes antigen. From this result, it would be speculated that the specific defect of cell-mediated immunity for *M. leprae* might be covered by other activated cellular immunity.

REFERENCES

1) Bryceson, A.D.M. (1981): Clinical, immunological and Epidemiological features of leishmaniasis. Humber, D. P. ed. Immunological aspects of leprosy, tuberculosis and leishmaniasis, Addis Ababa, Ethiopia, 32-38.

2) Buchanan, T., Dissanyake, S., Young, D.B., Miller, R.A., Acedo, J.R., Harnish, J.P., Khanolkar, S.R. and Estrada-Parra, S. (1983): Evaluation of significance of antibodies to phenolicglycolipid of *Mucobacterium leprae* in leprosy patients and their contacts, Int. J. Lepr., 51, 658-659

3) Furuya, M., Mimori, T., Gomez, E.A.L., De Coronel, V. V., Kawabata, M. and Hashigichi, Y. (1989): Epidemiological survey of leishmaniasis using skin test and ELISA in Ecuador, Jpn. J. Trop. Med. Hyg., 17, 331-338

4) Gaylord, H. and Brenann, P.J. (1987): Leprosy and leprosy bacillus; recent development in characterization of antigens and immunology of the disease. Ann. Rev. Microbiol., 41, 645-675

5) Hunter, S.W., Fujiwara, T. and Brenann, P.J. (1982): Structure and antigenicity of the major specific glycolipid of *Mycobacerium leprae*. J. Biol. Chem., 275, 15072-15078

6) Izumi, S., Maeda, M., Van Beers, S., Mdjid, B. and Kawatsu, K. (1993): Distribution of anti-LAM-B antibodies in leprosy patients and house hold contacts, 66 th Japanese leprosy Conference, p. 42

7) Izumi, S., Maekawa, M., Kawatsu, K. (1993): Distribution of Anti-LAM-B antibodies in leprosy patients and house hold contacts, Report of 26th Joint conference of Tuberculosis and Leprosy (U.S-Japan Cooperative Medical Science Program), 105-110

8) Ridley, D.S. and Jopling, W.H. (1966): Classifacation of leprosy according to immunity, Int. J. Lepr., 34, 255-273