

Prospective study of seroepidemiology: persistent seropositives developed leprosy

Khin Nwe Oo, Nwe Nwe Yin & Tin Tin Han

Immunology Research Division
Department of Medical Research (Lower Myanmar)

This study was carried out on the residents of Kyanbokone village, Nyaunglaybin Township, Bago Division and Natural Trisaccharide Phenyl Propionyl – Bovine Serum Albumin (NTP-BSA) ELISA was done on their sera. It was studied from May 2000 to September 2002. The IgM seropositives in this village were 74/265 (27.92%), 52/237 (21.94%) and 38/125 (30.4%) in the 1st, 2nd and 3rd years respectively. There were 17 and 8 subjects of 2 and 3 years persistent seropositives respectively. Most of the seropositives were household and neighbor contacts of old leprosy cases. Among 17 and 8 subjects of 2 and 3 years persistent seropositives, one each developed leprosy in 2001 and 2002 respectively in this village. When this serological test was carried out as a prospective longitudinal survey it can be used in detection of high risk subjects. Chemoprophylaxis would be needed to consider on persistent seropositives for not developing new cases of leprosy.

INTRODUCTION

Leprosy has been endemic in Myanmar since ancient days. In 1986, WHO Multi Drug Therapy (MDT) was introduced in Myanmar. At that time prevalence rate (PR) was 59.3/10,000 population. In 1990, prevalence rate of leprosy under MDT came down to 27.6/10,000. In 1991, there was a full integration of leprosy MDT program into Basic Health Service. The principle implementers were midwives of sub-rural health centers. In 1995, MDT service achieved 100% coverage by leprosy cases as well as in areas over the whole country. In 2002 December, prevalence rate of leprosy decreased to 1.04/10,000 [1].

In Myanmar, leprosy elimination (PR < 1/10000) was achieved at national level at the end of January 2003. Prevalence rates (PR 1.04/10000) of 70 townships mainly from Ayeyawady, Bago, Mandalay, Sagaing Divisions and Shan State are higher than elimination level. Up to June 2003, new case detection rate (NCDR) is more than

4/10,000 in 6 hyperendemic divisions and southern Shan State. NCDRs of Ayeyawady and Bago Divisions are higher than that of other 4 hyperendemic divisions in 2002 as well as in first half of 2003 [2].

Bago Division is located on the southern part of the country. It is 50 kilometers away from capital city, Yangon. It consists of 28 townships, 14 each in east and west regions. Total population in Bago Division is 4598736 in 2001. There are 1436 village tracts and 6296 villages. There are 1 leprosy specialist, 5 team leaders, 8 leprosy inspectors and 43 junior leprosy workers. Special focus Leprosy Elimination Campaign was done in December 2001 in West region and May 2002 in East region. The prevalence rate of leprosy in Bago Division was 2.3/10,000 in 2001 and the same in August 2002 (personnel communication, Leprosy Regional Officer, Bago Division).

Since 1999, our research group has been conducting a series of seroepidemiological

studies of leprosy in endemic pockets of Myanmar that will be useful both for sustaining leprosy elimination and decreasing the number of new cases in our community. Therefore this is one of the seroepidemiological studies carried out in Kyanbokone village, Nyaunglaybin Township, Bago Division as a prospective study from 2000 to 2002.

MATERIALS AND METHODS

The study was carried out targeting all the villagers in Kyanbokone village, Nyaunglaybin Township, Bago Division which is an endemic area of leprosy in Myanmar. The registered cases in Bago Division were 1070 in 2001 (669 multibacillary and 401 paucibacillary cases) and 1062 in August 2002. The new case detection rate was 1153 in 2001 and 785 in August 2002 (444 MB and 341 PB cases). MDT coverage was 100% at that time. The population of Kyanbokone village was 306 and there were 62 houses in this village. This study was carried out from May 2000 to September 2002. Ethical approval was obtained from the Ethical Committee of Department of Medical Research (Lower Myanmar). Blood from villagers was taken out using disposable needles and syringes in June 2000, July 2001 and June 2002. Sera were obtained by centrifuging with 3000 rpm for 10 minutes. They were kept in -20°C until further use.

Villagers were examined dermatologically by leprosy specialist, leprosy inspector and junior leprosy workers. Leprosy patients were classified as paucibacillary (PB) or multibacillary (MB) according to criteria of the WHO.

Sera from all of these residents were tested by enzyme linked immunosorbent assay for immunoglobulin M (IgM) antibodies to NTP-BSA antigen (kindly provided by Dr. Fujiwara, Nara University, Japan). The sensitivity of this test is 96% and 62% to

MB and PB cases respectively and the specificity is 96% [3]. The horse radish peroxidase-conjugated goat anti human IgM antibody (Dako, Denmark) was used as 2nd antibody and o-phenylenediamine (Wako, Osaka, Japan) as substrate. All results were expressed as optical density (OD) at 492nm in an ELISA reader of Stat Fax 3200 (Awareness Technology, Palm City, USA). Pooled sera from 10 multibacillary patients and normal apparently healthy subjects of National Blood Bank, Yangon were used as positive and negative control respectively.

RESULTS

The demographic situation of first, second and third years in Kyanbokone village are shown in Table 1. The leprosy situation in Kyanbokone village before this study was 1 case each in 1985, 1986, 1991, and 4 cases in 1999. In 1999, there were 3 PB cases and 1 MB. The anti IgM antibodies positivity in this village was 74/265 (27.92%), 52/237 (21.94%) and 38/125 (30.4%) in the 1st, 2nd and 3rd years respectively.

Table 1. The demographic situation of Kyanbokone village where NTP-BSA ELISA test done

Demographic situation	Kyanbokone village, Nyaunglaybin Township, Bago Division		
	1 st year (2000 June)	2 nd year (2001 July)	3 rd year (2002 June)
Population	306	304	286
Mean Age	27	28	22
Number Examined	265	237	125
Percent Examined	86.60%	77.96%	43.71%

Graphic presentation of the NTP-BSA ELISA with IgM antibodies of villagers' sera from Kyanbokone village (first, second and third years) is shown in Fig. 1.

Age specific distribution of anti IgM NTP-BSA antibodies positivity in this village is shown in Table 2.

Table 2. Age specific distribution of anti IgM NTP-BSA antibodies positivity in Kyanbokone village

Age range	Kyanbokone village, Nyaung laybin Township, Bago Division		
	1 st year (2000 June)	2 nd year (2001 July)	3 rd year (2002 June)
<9	112/47 ²	12/35	9/25
10 – 19	22/70	16/62	8/27
20 – 29	15/47	7/39	8/16
30 – 39	5/28	6/28	7/20
40 – 49	12/34	2/31	2/16
50 – 59	5/18	3/20	1/8
60-	3/21	6/22	3/13

N¹ = number of seropositives
 N² = number of tested subjects

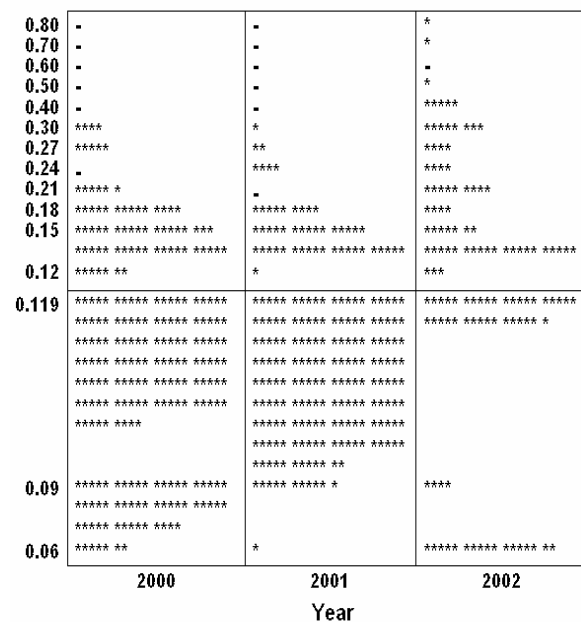


Fig. 1. Scattergram of the OD values of anti IgM, NTP-BSA antibodies titers of villagers' sera of Kyanbokone village

The horizontal line indicates the cut-off value between seropositives and seronegatives (The cut-off value is 0.120)

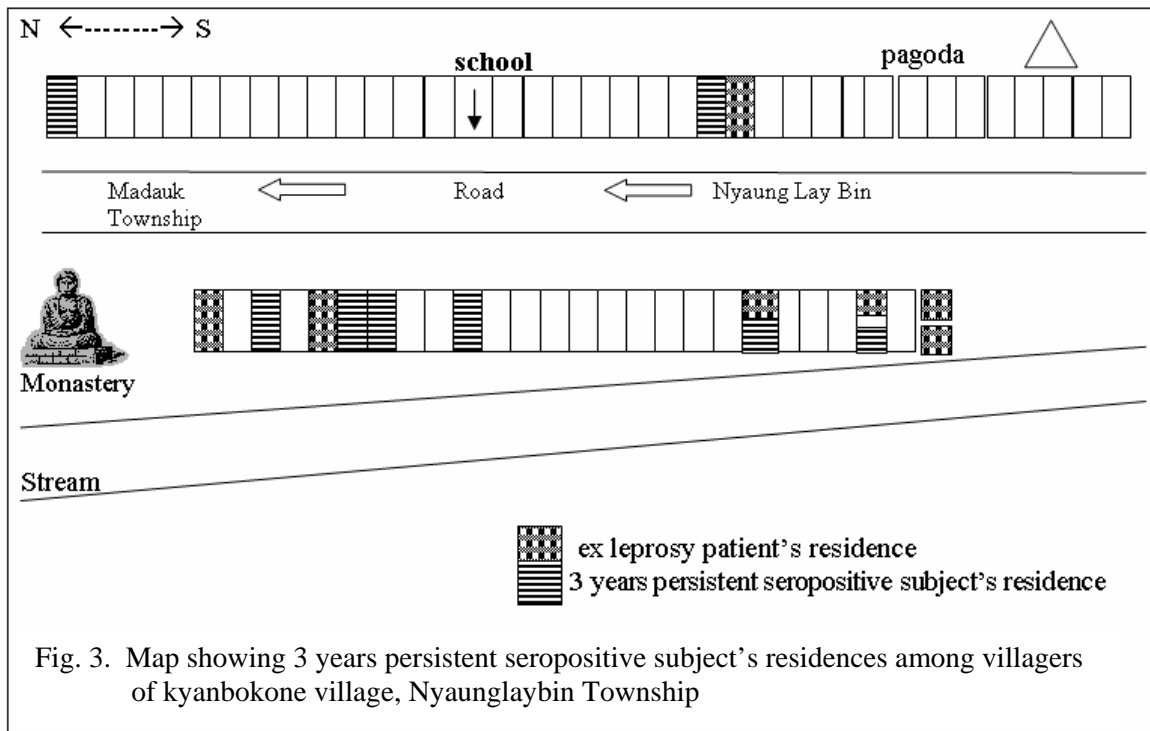
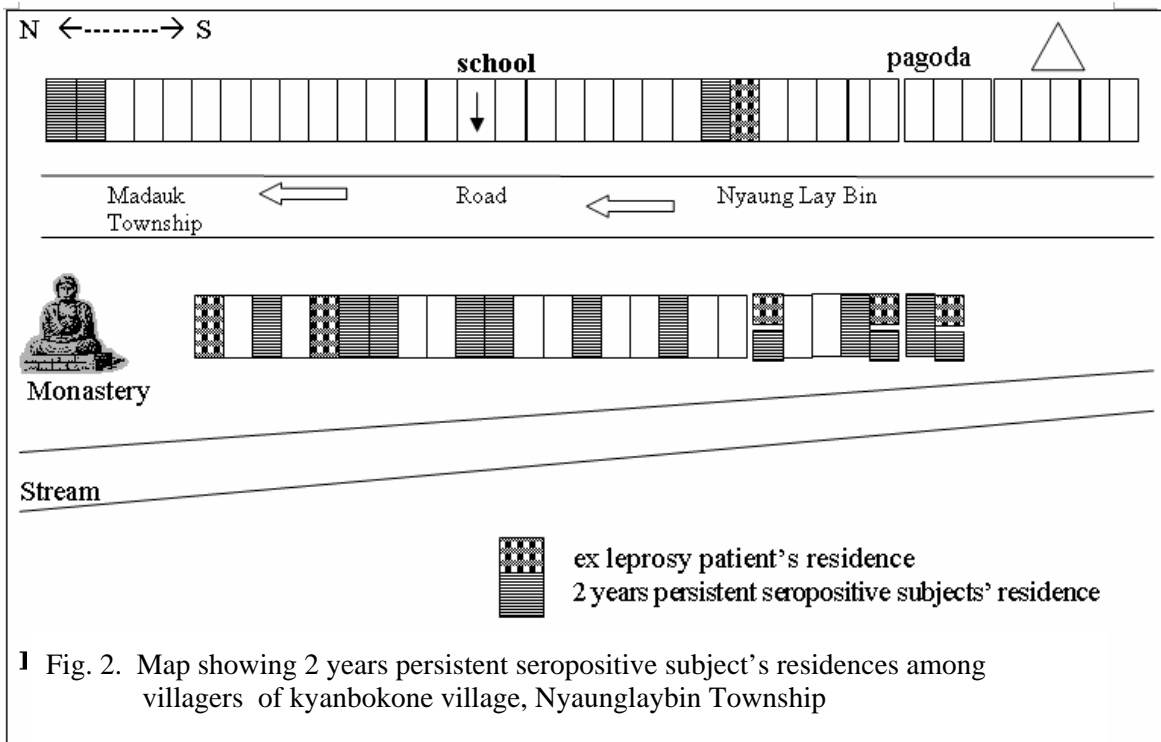
As shown in Figs. 2 and 3 most of the persistent seropositives were household and neighbor contacts of the old patients. There were 17 and 8 subjects of 2 and 3 years persistent seropositives respectively in our

study. In 2 years persistent seropositive subjects there were 2 subjects each in house no. 42 and 47. Among these 17 and 8 subjects of 2 and 3 years persistent seropositives, one each developed leprosy in 2001 & 2002 in our study period. The new case of 2001 was MB case and her residence was house no. 56. The new case of 2002 was PB case and her residence was house no. 60.

DISCUSSION

A positive ELISA test result cannot display a high predictive value for overt disease since infection is far more common than by clinically manifested cases. Thus, the test should be useful for screening populations or groups of individuals at risk and would screen out the small proportion that were seropositives for clinical examination [4]. Only a minority of persistent seropositive persons developed leprosy. These observations suggest that subclinical infection with *M. leprae* is common in endemic countries and that PGL-1 seropositivity is a marker of subclinical infection, with poor specificity for overt disease [5]. In this study, one each of 2 and 3 years persistent seropositives developed leprosy in 2001 and 2002 respectively.

This study reports on the serodiagnosis of leprosy infection in the prospective longitudinal community survey in high endemic village in Bago Division, Myanmar. As shown in graphic presentation of the NTP-BSA, ELISA with IgM antibodies of villagers' sera, some cases had high titre of antibodies. To sustain leprosy elimination and to reduce new case detection in high endemic areas, chemoprophylaxis would be needed to carry out. Therefore we would need to consider chemoprophylaxis on the persistent seropositive cases with the aim to prevent change to overt clinical cases.



ACKNOWLEDGEMENT

This study was supported by a grant from Nirvana NGO's group of Japan. We thank Dr. Fujiwara, Tokyo for gifting NTP-BSA antigen and Dr. Namisato and Prof. Kashiwabara, Tokyo for giving some supplies to carry out this research work.

REFERENCES

1. Kyaw Lwin. Road to elimination of leprosy in Myanmar, 50 years experience (1952-2002) Progress towards leprosy elimination in Myanmar (50 years journey)(1952-2002), 2003.
2. Kyaw Myint. Current situation of leprosy elimination in Myanmar and major challenges for research, 2004.

3. Gaylord A & Brennan P J Leprosy and the leprosy bacillus: Recent development in characterization of antigens and immunology of the disease. *Annual Review in Microbiology* 1987; 41: 645-675.
4. Gonzalez-Abreu E, Pon J A, Hmopez P, *et al.* Serological reactivity to a synthetic analog of
5. PGL-1 and the detection of in an area of low Endemicity. *Leprosy Review* 1996; 62: 4-12.
6. Baumgart K W, Britton D L, Mullins R J, Basten A & Barnetson R S. Subclinical infection with *M. leprae*: a problem for leprosy control strategies *Transactions of the Royal Society of Tropical Medicine and Hygeine* 1993; 87:412-418.