

**Seroepidemiology on extended contacts of new leprosy cases
in Nyaungdon Township**

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The contacts of the leprosy cases are risk groups to get acquire infection and develop disease after a long incubation period. Thus seroepidemiology was carried out on extended contacts of new leprosy cases in Nyaungdon Township, Ayeyawady Division. The prevalence rates of Ayeyawady Division and Nyaungdon Township were 1.52/10,000 and 1.8/10,000 respectively in 2002. There were 32 new leprosy cases (15 paucibacillary and 17 multibacillary) from September 2002 to August 2003. The study population was extended contacts of these new leprosy cases. The blood samples were collected from September to December 2003 after obtaining informed consent. Eight hundred and twenty nine (829) extended contacts were enrolled in this study. NTP-BSA ELISA test was carried out on their sera. Cut-off OD value was 0.120. Seropositivity rate of overall contacts was 309/829 (37.27%). In this study, seropositivity rate of overall contacts, household and neighbor contacts of both multibacillary (MB) and paucibacillary (PB) cases were similar. The seropositivity rate in children (40.35%) was more than the rate in adult contacts (36.49%), but it was not significant. The seropositivity rate of females (42.73%) was significantly increased compared to males i.e. 30.66%. Mostly mean antibody titer of household contacts (HC) was slightly more than neighbor contacts (NC) on both types. Those with high antibody level were much more likely to develop disease than those with low levels. Follow-up survey is needed to detect whether these contacts develop signs and symptoms of leprosy or have antibody titers persistently.

INTRODUCTION

In 1986 World Health Organization (WHO) Multi Drug Therapy (MDT) was introduced in Myanmar. At that time prevalence rate was 59.3/10,000 population. In 1990, prevalence rate of leprosy under MDT treatment 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 area over the whole country. In 2002 December, prevalence rate of leprosy decreased to 1.04/10,000 [1].

In Myanmar, leprosy elimination was achieved at national level at the end of January 2003. Prevalence rate of 70 townships mainly from Ayeyawady, Bago, Mandalay, Sagaing Divisions and Shan State is 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 other 4 hyperendemic divisions in 2002 as well as in first half of 2003 [2].

Among major infectious diseases, leprosy is probably least understood as to its epidemiology. Our knowledge on natural

history of the disease is also limited. This is largely due to the fact that causative microorganism of *M. leprae* has not been cultivated *in vitro*. Even sources of infection and mode(s) of transmission are still subjects of controversy, it is generally considered that human beings are the most important source of infection. On the other hand, many results of seroepidemiology have suggested that infection with *M. leprae* is far more common than overt disease [3, 4].

NTP-BSA ELISA was done on sera of residents of Kyanbokone village, Nyaunglaybin Township, Bago Division, Myanmar from May 2000 to September 2002. IgM seropositives in this village were 23.1%, 17.8% and 30.7% in 1st, 2nd and 3rd year respectively. There were 17 and 8 subjects of 2 and 3 years persistent seropositives respectively in this study. Most of the seropositives were household and neighbor contacts of old leprosy cases. Out of 17 and 8 subjects of 2 and 3 years persistent seropositives, 1 subject each developed leprosy. Therefore, serological test can be used in early detection of leprosy cases [5].

Only a minority of persistently seropositives developed leprosy. These observations suggest that subclinical infection with *M. leprae* is common in endemic communities and that NTP-BSA seropositivity is a marker of subclinical infection [6].

Previous studies in Myanmar were undertaken on whole population in pocket area (Kyanbokone village). Therefore this study was carried out on extended contacts (household and neighbor contacts of patients) of new cases of leprosy to know seroepidemiology among contacts of new leprosy cases in our community.

General objective of the study was to determine seroepidemiology on extended contacts of new cases in pocket area of leprosy. Specific objectives were (1) to choose one pocket area (with high prevalence rate, presence of new cases in recent years including multibacillary cases) (2) to

determine seroassay using Natural Trisaccharide Propionyl Phenyl-Bovine Serum Albumin (NTP-BSA) Enzyme Linked Immuno Sorbent Assay (ELISA) on extended contacts of new cases in the above area.

MATERIALS AND METHODS

This study was a community-based, cross-sectional study. The chosen township was Nyaungdon Township, Ayeyawady Division (prevalence rate was 1.8/10,000 in December 2002) and had 32 new cases including 17 multibacillary and 15 paucibacillary cases from September 2002 to August 2003. The new cases were from 6th and 11th quarter of Nyaungdon Township, Myoma, Chaunggyi, Samalauk, Thangyo, Sekkau, and Inma rural health centers. The study population was extended contacts of new leprosy cases. The samples were collected from September to December 2003. Specimen collection was undertaken by the investigators and leprosy field workers. Informed consent was obtained before collecting blood sample from the subject.

The blood was withdrawn using disposable needles and syringes. The samples were transported to Immunology Research Division, Department of Medical Research (Lower Myanmar). They were centrifuged at 3000 rpm x 10 mins and sera were kept in -20°C until further use. Blood samples from apparently healthy blood donors of National Blood Bank, Yangon were collected and used as controls. NTP-BSA ELISA test was carried out on villagers' and controls' sera. Cut-off value was mean + 2SD of OD of control blood samples.

NTP-BSA ELISA technique

ELISA microtitre plates (Fastec microplate U, Fujirebio, Inc.) with 96 U shaped wells were coated with 50 ul of 1 ug/ml concentration of soluble NTP-BSA antigen, incubated at 37°C for 2 hours and then placed at 4°C overnight. The antigen was diluted with carbonate-bicarbonate buffer to get final concentration. Next day, the

solution was sucked out and plates were then washed 3 times with phosphate buffer saline (PBS) containing 0.05% Tween 20 (PBST). 100 ul of blocking agent was added to each well and incubated at 37°C for one hour. It was washed with PBST for 3 times again. Fifty microlitre of test or control sera, diluted in 1:300 with dilution buffer were added to each well and incubated at 37°C for 1 hour. It was washed with PBST for 3 times again. Fifty microlitre of goat anti-human IgM conjugated to horse-radish-peroxidase, diluted in 1:1000 with dilution buffer were added to each well. It was incubated at 37°C for one hour. It was then washed with PBST for 3 times again. One-hundred microlitre of substrate solution (ortho-phenylene-diamine) were added to each well and incubated at 37°C for 15 min. Then stopping solution was added to stop reaction. Optical density (OD) was measured by ELISA reader using wavelength of 492 nm. Mean OD was calculated from duplicate results.

RESULTS

The prevalence rates of Ayeyawady Division and Nyaungdon Township were 1.52/10,000 and 1.8/10,000 respectively in 2002. There were 32 new leprosy cases (15 paucibacillary and 17 multibacillary types) in this township from September 2002 to August 2003. The new cases were from 6th and 11th quarter of Nyaungdon Township: Myoma, Chaunggyi, Samalauk, Mezali, Thangyo, Sekkau and Inma rural health centers.

The blood samples were collected from 32 new leprosy cases and 829 extended contacts. Extended contacts were household contacts, neighbor I (either side of patient's house), neighbor II (either side of neighbor I) and neighbor III (either side of neighbor II). Among 829 contacts, there were 403 (48 household and 355 neighbor) contacts of PB cases and 426 (57 HC and 369 NC) contacts of MB cases.

Seropositivity rates by rural health centers are shown in Table 1. It was the highest in Inma RHC in which there were 11 (6 PB and 5 MB) new leprosy cases. The highest seropositivity rate on HC of PB and MB was 5/6 (83.33%) and 3/3 (100%) respectively. The rate of HC was more than NC on both types of leprosy. Seropositivity rates of overall paucibacillary (PB) and multibacillary (MB) contacts were 152 / 403 (37.72%) and 157/426 (36.85%) respectively. The rates of household contacts of PB and MB were 23/48 (47.92%) and 26/57 (45.61%) respectively and neighbor contacts of them were 129/355 (36.34%) and 131/369 (35.5%) respectively.

Table 1. Seropositivity rates of extended contacts by RHCs (Rural Health Center) in Nyaungdon Township

RHC	No. of new cases	Seropositivity rate
Myoma & Chaunggyi	5 (3PB, 2MB)	36/117 (30.77%)
Samalauk	4 (1PB, 3MB)	49/131 (37.40%)
Mezali	5 (1PB, 4MB)	35/110 (31.82%)
Thangyo	4 (2PB, 2MB)	46/117 (39.32%)
Sekkau	3 (2PB, 1MB)	30/77 (38.98%)
Inma	11 (6PB, 5MB)	113/277 (40.79%)

PB = Paucibacillary type of leprosy

MB = Multibacillary type of leprosy

Table 2. Seropositivity rates of extended contacts by type of contacts in Nyaungdon Township

Type of contacts	PBHC	PBNC	MBHC	MBNC	Seropositivity rate
Overall contacts	23/48 (47.92%)	129/35 (36.34%)	26/57 (45.61%)	131/369 (35.5%)	309/829 (37.27%)
Adult contacts	19/34 (55.88%)	100/280 (35.71%)	19/45 (42.22%)	102/299 (34.11%)	240/658 (36.49%)
Children contacts	4/14 (28.57%)	9/75 (38.67%)	7/12 (58.33%)	29/70 (41.43%)	69/171 (40.35%)
Male contacts	9/22 (40.91%)	51/163 (31.29%)	8/26 (30.77%)	47/164 (28.66%)	115/375 (30.66%)
Female contacts	14/26 (53.85%)	78/192 (40.63%)	18/31 (58.06%)	84/205 (40.98%)	194/454 (42.73%)

PBHC = Household contacts of paucibacillary leprosy

PBNC = Neighbor contacts of paucibacillary leprosy

MBHC = Household contacts of multibacillary leprosy

MBNC = Neighbor contacts of multibacillary leprosy

Children vs. Adult: $\chi^2=0.39$ $p>0.05$

Females vs. Males: $\chi^2=5.90$ $p<0.01$

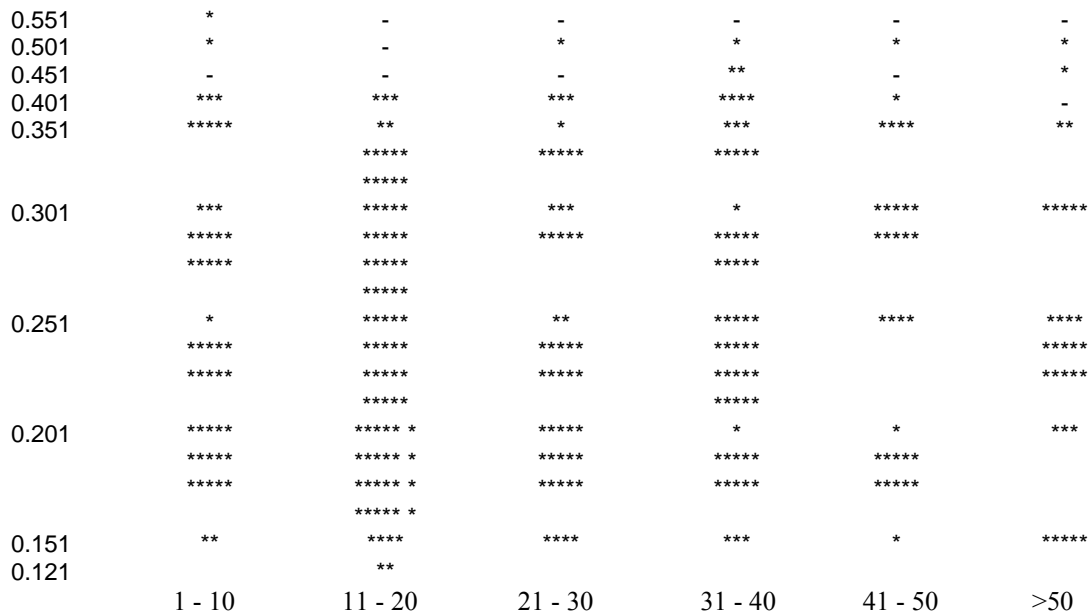


Fig. 1. Scattergram showing seropositives among extended contacts of new cases of leprosy by age range in Nyaungdon Township
Cut-off point = 0.120 (absorbance at λ 492 nm)

Table 3. Mean antibody titers (IgM index) by type of leprosy

RHCs	PBHC	PBNC	MBHC	MBNC
Myoma & Chaunggyi	3.117	1.717	1.017	1.208
Samalauk	2.10	1.233	2.217	1.667
Mezali	1.817	1.625	2.275	2.15
Thangyo	1.733	1.80	2.092	1.858
Sekkau	2.025	1.95	1.625	1.692
Inma	2.542	2.308	2.267	2.033
Inma	2.792	2.592	2.833	2.25

PBHC = Household contacts of paucibacillary leprosy
 PBNC = Neighbor contacts of paucibacillary leprosy
 MBHC = Household contacts of multibacillary leprosy
 MBNC = Neighbor contacts of multibacillary leprosy
 IgM index = Mean anti IgM antibody titers /cut-off value

Seropositivity rates by type of contacts are shown in Table 2. Overall seropositivity rate was 309/829 (37.27%). It was seen that seropositivity rate of children 69/171 (40.35%) were more than adult contacts 240/658 (36.49%). The difference between children and adult contacts was not statistically significant ($\chi^2=0.39$, $p>0.05$).

Seropositivity rate of females 194/454 (42.73%) was more than males 115/375 (30.66%) and the difference between females and males was statistically significant ($\chi^2=5.90$, $p<0.01$).

Mean antibody titer by type of leprosy was shown in Table 3. Mostly mean titer of HC was slightly more than NC on both types of leprosy.

In this study, cut-off point of OD was 0.120 (Khin Nwe Oo *et al.*, 1999). If high increased antibody titer was assumed as $OD>0.400$, there were 9 highly seropositives in this study, only 1 was HC of MB case, 2 and 6 were NC of MB and PB cases respectively.

Fig. 2 shows seropositives among extended contacts of new cases of leprosy by age range in this study.

DISCUSSION

Clinical evaluation fails to identify persons with subclinical infection, those without clinical evidence of infection, including some early multibacillary (MB) cases [8]. These (high risk group) individuals with early MB disease may be highly infectious for some years before detection and pose a major strategic problem for leprosy control [9]. This NTP-BSA ELISA test could be used in serodiagnosis of subclinical infection and in early diagnosis of disease. It was also shown in previous study of Khin Nwe Oo and coworkers [5].

When household contacts of multibacillary leprosy cases were detected with anti-D-BSA and anti PGL-1 assays seropositivity rate was 3/28 (10.7%) [8]. Extensive studies have used the determination of anti-D-BSA IgM to screen contacts on endemic areas giving positivity rates of around 20% [10]. In this study the seropositivity rate of overall contacts using NTP-BSA ELISA was 309/829 (37.27%). Therefore it was shown that this study gave more seropositivity rate on contacts than other studies.

As shown in Table 1, the seropositivity rate was the highest in Inma rural health center (RHC) in which there were more new leprosy cases than other RHCs.

The highest seropositivity rate on household contacts of PB and MB was 83.33% and 100% respectively. Therefore the difference was only 16.67%. In this study the seropositivity rates of household and neighbor contacts of both PB and MB cases were similar. It is generally assumed that antibody levels are associated with the intensity of exposure to *M. leprae* [11]. The seropositivity rates in contacts were similar in both types of leprosy, suggesting a uniform risk of exposure in this population in high prevalence area.

As shown in Table 2, the seropositivity rate was higher in children than adult contacts. It showed a similar age related pattern as demonstrated before [4]. Significantly high seropositivity rate among females was observed and it has been reported in a number of other studies [8, 11]. Higher innate IgM level among females compared to males is the simple explanation for this phenomenon [12].

As shown in Table 3, mean antibodies titer had not much difference between MB and PB contacts. There was also no substantial difference between household and neighbor contacts in other study [8].

The extended contacts having highly increased antibodies were present in both

MB and PB contacts, as well as in both household and neighbor contacts. Those with high antibody levels were much more likely to develop the disease than those with low levels. Therefore follow up will be needed to know whether they develop the disease or persist of their antibodies A., B. A.B., C.B. *et al.*

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