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Effect of chronic lead exposure on children of Yangon, Myanmar (2): Physical growth and development

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A cross-sectional comparative study was undertaken to explore the effect of chronic low-level lead exposure on the growth and development of children, 82 children in Yangon, who were chronically exposed to lead and 82 non-exposed children who were sex and age matched with the exposed subjects were studied. Blood lead was determined as the indicator for lead exposure. Mean blood lead levels in exposed children were 34.85 ± 9.99, 32.5 ± 18.23 and 36.44 ± 22.37 µg/dl for below 3 years, 3-6 years, and above 6 years age groups respectively while the corresponding values in the non-exposed children were 11.33 ± 8.55, 13.17 ± 8.9, and 15.03 ± 8.7 µg/dl. The differences were statistically significant ($p<0.001$ for all age groups). Percentage of children with height-for-age less than (-3SDs) of National Center for Health Statistics (NCHS) standard was found in 12% of the exposed children but none in the non-exposed ones. Percentage of children with weight-for-age below 3SDs was found in 10% of the exposed children but only 2% among the nonexposed group. Results from the Denver's Developmental Screening Test showed that 23.5% of the exposed children were suspected of having developmental retardation while in the corresponding value in nonexposed children was 12.5%. Present study thus highlighted the detrimental effect of chronic low-level lead exposure on the growth and development of children.

INTRODUCTION

The growth-retarding effects of blood lead concentration have not been well defined. Although the adverse effect of overt plumbism on physical growth has long been recognized [1, 2], the effect of low-level lead exposure on physical growth was not well documented. It was first explored by Schwartz *et al*. using data from the National Health and Nutrition Examination Survey (NHANES) II of 1976-1980 [3]. The NHANES II data for 2695 children 7 years old (included Non-Hispanic Whites, African-Americans, and Mexican-Americans) indicated that blood lead level (range = 4-35 µg/dl) was a statistically significant predictor of children's height, weight, and chest circumference, with control for age, race, sex, and nutritional covariates. However, the cross-sectional nature of the NHANES II survey limited causal inference regarding the relationship.

The results of subsequent studies have been inconsistent. A retrospective study of the growth of 54 Hispanic children from birth to 48 months of age suggested a negative correlation between weight gain and higher blood lead between 15 and 24 months of age [4]. Two longitudinal studies on the African-Americans and non-hispanic Whites did not find any significant association between blood lead and physical growth [5, 6]. In another longitudinal study on the German children, covariate-adjusted heights at 15 and 33 months of age were negatively associated with postnatal blood

lead concentrations [7]. Indeed, with control for other variables, including the child's medical history, dietary history, behavior, tobacco smoking of parents, and socio-demographic factors, a study of Danish children showed an inverse association between tooth lead and height [8]. As the similar kind of studies have not been conducted in Myanmar yet, the present study was carried out to explore the impact of chronic low-level lead exposure on the children of Myanmar in terms of physical growth and development.

MATERIALS AND METHODS

It was a cross-sectional comparative study on the children of Myanmar (1 to 12 years old) whose parents were occupationally exposed to lead in the selected townships of Yangon namely New Dagon (South), Hlaingtharyar and Thingangyun, conducted from December 2001 to July 2002. The three townships were selected as the former two townships have Industrial Zones where the battery factories and small-scaled battery shops were located. In Thingangyun Township, there is one ward called "Kyiphwarye" where almost the whole community was engaged in lead smelting business. From each township, list of occupations related to use of lead, were obtained from the Office of Industrial Zones (New Dagon and Hlaingtharyar) and Township Development Committee. According to the list, battery factories, battery shops and lead smelting sites were visited together with the township medical officer and staff from the Industrial Zones/ Township Development Committee. During the visits, lists of workers' children between 1 to 12 years of age were obtained. Children of the workers of Industrial Zones lived in separate areas while children of the workers from Thingangyun lived adjacent to the work site. All children between 1 to 12 years of age listed were recruited for the study.

Parents were given complete information regarding the nature of the study and only the children of those parents who gave voluntary consent were participated in the study. The parents were interviewed for basic demographic information, past and present occupational history, past medical history, and personal hygiene. All children were medically examined, including medical history, anthropometric measurements (height and weight) laboratory tests (blood lead, urinary coproporphyrin) and developmental screening with Denver's Test was done on under-six-year children. Comparable non-exposed subjects from the same selected townships were also assessed with the same procedure.

Determination of blood lead

Blood lead determination was done by dithizone extraction method as described by the National Institute of Occupational Safety and Health in 1987 [9].

Physical growth

Subjects' weight and height were measured (in light clothes and barefoot) to 0.1 kilogram and 0.1 centimeter, respectively, with use of bathroom scale and steadiometer.

Developmental screening test

The development of the children was screened with Denver's Test which is designed to compare a given child's performance on a variety of tasks to the performance of other children of the same age. It can be interpreted as Normal, Suspect and Untestable development of the children between birth and six years of age [10].

Statistical analysis

Comparison of the blood lead levels between the exposed and non-exposed children was made by employing Student's "t" test. For comparison of the height-for-

age and weight-for-age values between the two groups chi- square test was applied.

RESULTS

Table 1 presents the general characteristics of the study population. Exposed and non-exposed children were comparable in age, family income and educational attainment of parents.

Table 1. Characteristics of the study population

Characteristics	Exposed	Non-exposed
Total no. of children	82	82
Male	54	44
Female	28	38
Age (mean ± years)		
< 3 yr	1.9 ± 0.81 (14)	2.0 ± 0.90 (14)
3 – 6 yr	4.5 ± 1.06 (32)	4.7 ± 1.12 (34)
>6 yr	9.3 ± 2.21 (36)	9.22 ± 2.13 (36)
Family income (kyats/month) (mean ± S.D.)	16975 ± 153.33	16500 ± 63.58
Father's educational attainment (avg. standard passed)	9.1 ± 3.4	9.07± 2.7
Mother's educational attainment (avg. standard passed)	5.5 ± 2.2	5.2 ± 3.0

Figures in parenthesis show number of samples

Table 2. Comparison of mean blood lead level between exposed and non-exposed children

Age (Year)	Blood lead level (μg/dl) (n)		
	Exposed (82)	Non-exposed (82)	P value
< 3	34.85 ± 9.99 (14)	11.33 ± 8.55 (12)	< .001
3 – 6	32.5 ± 18.23 (32)	13.17 ± 8.92 (34)	< .001
> 6	36.44 ± 22.37 (36)	15.03 ± 8.72 (36)	< .001
Total	34.92 ± 5.32	12.98 ± 8.75	<0.001

Figures in parenthesis show number of children

Table 2 presents the mean blood lead levels of exposed and non-exposed children. For all age groups, exposed children had significantly higher blood lead levels than the non-exposed. Differences were statistically significant. For all age groups, the blood lead levels of exposed children were higher than the cut-off point 15 μg/dl, while in the non-exposed group, children less than 6 years old had <15 μg/dl blood lead level and only in >6 year old group that blood lead level was in borderline.

Comparison of height for age and weight for age of exposed and non-exposed children are shown in Table 3. Percentage of children with height-for-age less than (-3SD) was found in 12% of the exposed children but none in the non-exposed. The differences were statistically significant ($p<0.001$). Although majority of the children from both groups were found to be in the more than (-2SD) category, higher percent (10% vs 2%) of exposed children had weight for age less than (-3SD). The differences were statistically significant ($p<0.005$).

Table 3. Comparison of height for age between exposed and non-exposed children

	Exposed % (no)	Non-exposed % (no)	P-value
Height for age			
More than -2 S.D*	64 (52)	78 (64)	0.04
Between (-2 S.D) to (-3 S.D)*	24 (20)	22 (18)	0.71
Less than (-3 S.D)*	12 (10)	0	0.001
Weight for Age			
More than -2 S.D*	54 (44)	71 (58)	0.02
Between (-2 S.D) to (-3 S.D)*	36 (30)	27 (22)	0.18
Less than (-3 S.D)*	10 (8)	2 (2)	0.05

*NCHS (1983)

Table 4. Developmental screening of exposed and non-exposed children

Category	DENVER Interpretation	Number of children	%
Exposed	Normal	25	73.5
	Untestable	1	3.0
	Suspect	8	23.5
Non-exposed	Normal	28	87.5
	Suspect	4	12.5

Results of the developmental test are presented in Table 4. The screening test showed that higher percent of exposed children were suspected of having developmental retardation than the non-exposed children.

DISCUSSION

There are few published investigations on the relationship of blood lead and growth. Some investigators indicate that children with blood concentrations between 2.40 and 2.88 umol/L are characterized by short stature when compared with standards of height [11, 12]. On the other hand, other studies report that children who experienced severe lead poisoning did not differ in stature from their controls who happen to be their siblings [13]. Experimental studies do show that neurobehavioural deficits, organ pathology, and weight deficits in rats are evident at blood lead concentrations that range from 8.0 to 24.0 µg/dl[14].

The present is the preliminary study to find the blood lead profile of children whose parents were occupationally exposed to lead and to determine the effect of chronic lead exposure on the growth and development of children. According to the Center for Diseases Control's (CDC) lead values, blood lead level of more than 10 µg /dl in a child is considered high. Results of the present study implied that all children whose parents were exposed to lead from their occupation had high blood lead levels and thus run the risk of chronic lead poisoning. They had significantly higher blood lead levels than their non-exposed counterparts. As both groups of children resided in the same community and comparable in age and ***socioeconomic status*** the differences in the biochemical indicators most probably reflect the parents' exposure to lead. Finding of high blood lead levels in certain percent of non-exposed children might probably due to living in the community where environmental exposure to lead was present.

Findings of the present study is in accord with the others as percentage of having less than (-3SD) height for age and weight for age values of NCHS standard were significantly more among the exposed children than the non-exposed. This finding, although, showed association between under-nutrition and increased blood lead level, is not conclusive evidence of the detrimental effect of chronic lead exposure on the physical growth of children, as dietary and other assessments related to nutritional status had not been conducted. But the findings emphasized the need to conduct more research in this aspect and for appropriate precautionary measures to be undertaken.

The results of the Denver's Developmental Screening Test indicated that, exposed children might be having developmental retardation. As the nature of the test is screening only, it could not be drawn into conclusion, but pointed out the need for further testing by more definitive tests.

The present study demonstrated that children of the parents who were involved in occupation exposed to lead were causing chronic lead exposure to the workers' children as evidenced by the blood lead levels. This exposure might also be exerting detrimental effects on the health of the children as shown by the anthropometric measurements and developmental screening test and more research should be devoted in this context to have conclusive evidence.

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Genotyping of *Mycobacterium leprae* on the basis of the polymorphism of TTC repeats for analysis of leprosy transmission

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The polymorphism of TTC repeats in *Mycobacterium leprae* was examined using bacilli from skin slit of leprosy patients attending at Central Special Skin Clinic, YGH and nasal swabs of their contacts to elucidate the possible mode of leprosy transmission. It was found that bacilli with different TTC genotypes were distributed among same household contacts and also harbored bacilli in patients were different TTC genotype from that harbored by the contacts. Genotypes of TTC repeats were found to differ between husband under treatment and his wife and also mother under treatment and her sons living in same house. These results revealed the possibility that in addition to exposure via the presence of a leprosy patient with a multibacillary (MB) infection who was living with family members, there might have been some infectious sources to which the residents had been commonly exposed outside the dwellings. A limited discriminative capacity of the TTC polymorphism in the epidemiological analysis implies the need of searching other useful polymorphic loci for detailed subdivision of clinical isolates. It was found that TTC genotype of bacilli harbored by household contacts was different with the TTC genotype by index cases. It was seen in the resident where TTC genotype was different in husband (index case) and his wife (HC) and the mother (index case) and her sons (HC). These results revealed that whether the family members get transmission either from their MB index cases or from outside the dwellings.

INTRODUCTION

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* infection. It has long being believed that the source of infection is untreated multibacillary leprosy patients. It has also been predicted that multidrug therapy (MDT) with strong bactericidal antibiotics (such as rifampicin) would reduce the source of infection and consequently interrupt further transmission to others. However, the number of new cases has shown no substantial decline. It is reported that about 600,000 to 700,000 new cases are continuously found in the world every year [1], which suggests that the transmission of leprosy bacilli still occurs, especially in countries of endemicity. Elucidation of the mode of transmission would be essential to reduce new cases detection rate. The differentiation of strains of leprosy bacilli by genomic polymorphism might be of great value in efforts to understand the mode of transmission of the disease. The range of molecular techniques for epidemiological analysis has expanded in recent years, and there are now many genotypic methods that allow a high level of discrimination between bacterial strains. Restriction fragment length polymorphism analysis, which is the method most widely

used for molecular epidemiology of tuberculosis, is not applicable for leprosy. *M. leprae* can not be grown in artificial medium, and almost no divergence was found by this finger printing assay [2]. Shin *et al*. discovered a genomic divergence of *M. leprae* by the variation of TTC repeats [3] and subdivided 34 isolates into 15 subtypes. Genotyping according to the TTC repeats for fragments amplified by PCR seemed to be feasible for molecular epidemiological analysis of leprosy transmission. A previous study by Saeki et al. revealed that *M. leprae* existed on the surface of nasal cavities of residents in areas of endemicity [4].

Here, we report the distribution of different TTC genotypes of *M. leprae* among family members of each household and inconsistent genotypes obtained from patients and their family members in the same dwelling. The results strongly supported the previously proposed hypotheses on the existence of an infectious source(s) other than that of patients living with family members.

MATERIALS AND METHODS

Samples from patients

To clarify whether the TTC genotype in one patient varies or not, genotypes of the bacilli obtained from various lesions of one patient were compared. Slit-skin smear samples from 45 lesions of 22 patients from Central Special Skin Clinic, YGH were obtained. Samples were collected in the same manner as is used for routine slit-skin smear testing for bacterial index examination.

The sample on the disposable surgical blade was soaked in 70% ethanol and kept at room temperature until use. The sample was removed from the blade and collected as a pellet by centrifugation at 10,000 rpm for 20 min in 70% ethanol and then washed with phosphate-buffered saline. The template was prepared by treatment with lysis buffer, and then the TTC genotype was examined.

Samples from patients and their contacts (who develop new case later) in the same dwelling

TTC genotypes of the bacilli from patients and their contacts (who develop new case later) living in the same dwelling were examined.

- Case 1 was MB case and his son developed as new case later.
- Case 2 was the same as Case 1 in another house.
- Case 3 was also MB case and after 10 months of MDT his daughter developed new case.
- Case 4 was also MB case and after 9 months of MDT his brother developed new case (Table 3).

Skin slit samples were collected from at least two lesions of each patient. The genotype of each isolate was examined as described below.

Samples from household contacts

TTC genotypes of the bacilli from nasal swab specimen of 100 household contacts (HC) were examined. HC were defined as persons sleeping during the night under the same roof. Nasal swabs were taken by introducing cotton tip swabs (sterilized *JCB* MENTIP, Japan) 2-3cm into each nostril successively, and rubbing gently on the lateral and median sides of each cavity. Swabs were immediately chilled and transported to the Immunology Research Division, DMR (L M) and analyzed.

Preparation of template DNA and sequencing analysis

Templates from nasal swab materials and slit-skin samples were prepared by treatment with lysis buffer at 60°C overnight as described in Klatser *et al*. [5], TTC repeat regions were amplified by PCR

with the primers indicated by Shin *et al.* [3]. Copy numbers of TTC repeats were examined by the direct sequencing of the PCR products. Briefly, the regions flanking TTC repeats were amplified using a G mixture and a FailSafe PCR system (EPICENTRE, Madison, Wis.). DNA samples for sequencing were recovered with a MinElute gel extraction kit (QIAGEN GmbH, Hilden, Germany) after electro-phoresis of PCR products. Samples were sequenced with a BigDye terminator cycle sequencing FS Ready Reaction kit (Perkin-Elmer Applied Biosystems, Norwalk,Conn.) and an ABI Prism 310 genetic analyzer (Perkin-Elmer). The nucleotide sequences obtained were analyzed using DNASIS software (Hitachi Software Engineering, Yokohama, Japan). The forward primer was used in all sequencing reactions, since the nucleotide sequences of interest detected by the reverse primer were deduced to be identical with those detected by the forward primer.

Ethical approval

Informed consent was obtained from all subjects. The study was approved by the Institutional Ethical Review Committee of Department of Medical Research (Lower Myanmar). Bacillary samples of nasal swabs and slit-skin smears were collected after informed consent was obtained.

RESULTS

Genotype of the bacilli from the nasal swab samples

Of 92 dwellings, there were 18 houses in which 30 (33%) individuals carried the bacilli on the surface of their nasal cavities. Residents in these houses harbored different TTC genotypes from each other; their TTC genotypes were 9, 11, 12, 13, 14, 15, 16, 17, 21, and 22 repeats. The TTC repeats of the bacilli from the new MB case consisted of 11 copies, but the bacilli from his family contacts showed 14 and 17 copies. The TTC repeats of the bacilli from PB patient showed 21 copies but bacilli from his HC showed 15 copies. The TTC repeats of the bacilli from another new MB case consisted of 13 copies, but the bacilli from his family contacts showed 13, 16 and 9 copies (Table 1). Among the dwelling, the most predominant genotype was 16 copies of TTC repeats and the 2nd dominant type was 14 copies of TTC repeats (Table 2).

Table 1. TTC genotypes of *M. leprae* detected from the skin and surfaces of nasal mucosa of patients and surfaces of nasal mucosa from residents living in the same house

Sr. No.	Leprosy patients (Type)	Contacts (Relationship)	TTC genotype (Slit skin)	TTC genotype (Nasal swabs)
1	MB		11	15
2		Wife	-	14
3		Son	-	17
4	PB		21	18
5		Grandmother	-	15
6	MB		16	15
7		Son	-	16
8		Son	-	15
9	MB		13	16
10		Daughter	-	13
11		Daughter	-	16
12		Son		9

MB = Multibacillary
PB = Paucibacillary

Table 2. Frequency of each genotype observed in patients and household contacts

No. of repeats	Genotype frequency		
	Patient's lesions	Nasal mucus	Total
9	2	1	3
11	2	1	3
12	6	4	10
13	6	6	12
14	4	9	13
15	4	8	12
16	11	12	23
17	2	4	6
21	2	3	5
22	6	4	10
Total	45	52	97

Genotype of the bacilli in the lesions

From all 22 patients, 45 samples of different lesions showed identical genotypes. The most dominant genotype has 16 copies of TTC repeats in these patients. The other genotypes (number 9, 11, 12, 13, 14, 15, 16, 17, 21 and 22 copies of TTC repeats) were detected. The frequency of each TTC genotype observed in samples from lesions of the patients and the nasal cavities of the residents is shown in Table 2.

Comparison of TTC genotypes among patients in a dwelling

The TTC genotypes of *M. leprae* of index and secondary cases were compared. The genotypes of patients (index cases) and son (secondary case) harbored the bacilli with 13, 22, copies and 9, 17, copies of TTC repeats respectively in 2 household in this study. In case 3 who was MB case harbored bacilli with 11 copies of TTC repeats, after 10 months of MDT his daughter developed as secondary case and harbored bacilli with 14 TTC repeats. Another case 4 of household cases of two brothers showed different TTC genotypes (15 and 16 TTC repeats) within the family (Table 3).

Table 3. TTC genotypes of *M. leprae* obtained from household leprosy cases

Case No.	Patient (TTC genotype) in supposed index case	Patient (TTC genotype) In same HHC*
1	Father (13)	Son (9)
2	Father (22)	Son (17)
3	Mother (11)	Daughter (14)
4	Older brother (16)	Younger brother (15)

HHC* = House Hold Contact

DISCUSSION

Elucidation and understanding of the source and the routes of transmission of *M. leprae* are essential in developing measures to prevent an infection. Previous sero-epidemiological studies indicated wide-spread *M. leprae* infections within a population [6, 7, 8, 9], and studies by PCR on the distribution of the bacilli also found that many individuals in areas in which leprosy is endemic carried *M. leprae* on the surface of their nasal cavities [5, 4, 9].

These studies suggested the presence of an infectious source other than that of a patient within the same dwelling. The aim of this study was to clarify microbiologically whether or not MB cases in the same dwelling represent the main source of infection. Establishing a methodology to discriminate the isolates of *M. leprae* is fundamental for these purposes. Although many attempts have been made to subtype *M. leprae* isolates by genomic divergence [10, 11, 2], no useful methods for epi-demiological analysis have been developed. Recently two genomic polymer-phisms successfully discriminated isolates of *M. leprae* [12, 3]. One of the authors (M. Matsuoka) discovered that *M. leprae* isolates could be divided into two subtypes on the basis of the polymorphism in the *rpoT* gene.

The geographical distribution of each genotype in the world was biased and seemed to be related to prehistoric movement of the human race [12]. Nevertheless, the genomic diversity of the *rpoT* cannot be used for epidemiological tracing of the transmission of leprosy bacilli. Genotyping to compare diversity of short-tandem-repeat loci on the basis of PCR is feasible for molecular epidemio-logical analysis, since *M. leprae* is not cultivable and shows very low levels of diversion in genomic DNA [13]. Variety in the copy numbers of TTC repeats can be used to classify *M. leprae* into a considerable number of subtypes and discriminate isolates for each leprosy case.

It is reasonable to assume that if the index case in the same dwelling is the source of infection, the genotypes detected in the house should be identical among the

household members. In this study, various types of TTC genotypes were detected from nasal mucosa of the HHC.

However, our results clearly demonstrated that there were families with different TTC genotypes of *M. leprae* on the surface of nasal cavities among the residents in the same dwelling. Therefore, the results of the investigation suggest that these residents are contaminated by bacilli with different genotypes. No variations in genotype among the isolates obtained from various lesions in the same patient were shown. This result consequently enables comparisons of the genotypes of bacilli obtained from different patients.

We had identified the existence of TTC genotypes of *M. leprae* that differed between the newly detected family contacts and the supposed index case patient. These results strongly suggest that the bacilli did not originate from a single patient in the dwelling and also indicate the exposure of the family members to infectious sources out of the dwelling. Previous seroepidemiological studies suggested that for the majority of cases, the possible source of infection might be in the environment rather than in direct contact with leprosy patients [6,7,8]. The findings by PCR, which revealed the wide distribution of the bacilli among the residents in areas of endemicity, also indicated that the transmission of the bacilli was not only from the leprosy patients [5, 4, 9].

The present study strongly supports these assumptions respecting the infectious source(s). Although many epidemiological observations indicated that the household contact was the risk factor for the development of leprosy [14, 15], on the other hand, many new cases had unknown source of infection [14]. Therefore, the source of the secondary case is not only from his/her household. The tendency seen of the accumulation of patients in some families might be attributed to other conditions such as susceptibility to leprosy infection, which is related to genetic predisposition as well as to acquired factors [16]. Two groups of the household leprosy cases showed apparently different TTC genotypes between a father and his son, mother and daughter and among brothers.

The inconsistency of the genotypes between *M. leprae* isolates obtained from household cases of patients living in the same dwelling clearly indicates that these patients are not always the source in infections of the other family members. Though the members of the other groups of leprosy cases showed the same genotype, whether those people were truly infected by the patient in the house was unclear. The presence of the same genotype in two cases doesn't necessarily imply the infection was transmitted from a patient to family contacts, for some TTC genotypes, such as those of 10 and 13 repeats, were widely distributed in the areas.

Other polymorphisms which can discriminate within a given TTC genotype are needed to elucidate this problem. Better epidemiological analysis could be done by the combination of various genotyping techniques. However, TTC genotyping enabled the subtyping of *M. leprae* into more types than *rpoT* genotyping. It is expected that other short polymorphic-tandem-repeat loci exist in *M. leprae* genome, in similarity tothose observed in investigations of *M. tuberculosis* [17]. A combination with genotyping using other polymorphisms might be a useful tool for precise epidemiological analysis.

Other genotyping measures depending on other short-polymorphic-tandem-repeat loci are required. The frequency of 24 or 25 TTC repeats was the highest in the previous study, which examined *M. leprae* isolates obtained in Cebu, Philippines [3]. Bacilli with 10 copies of TTC repeats were most frequently isolated in the present study, and the bacilli with large numbers (such as 37) of TTC repeats were not detected (Table 3).

It is of interest to compare the frequencies of each genotype in different areas, since the

results of a previous study indicated that the spread of the bacilli with specific genotypes was consistent with migration of some human groups [12]. The evidence resulting from the present molecular epidemiological study indicated the existence of an infectious source other than patients in the same dwelling. Wide distribution of the bacilli among residents [5,4,9] and a high positive ratio of anti-PGL-1 antibody among healthy residents [7,8] suggested that the bacilli existed in certain sources to which people were commonly exposed. Taking these results into consideration, the environment seems to be the most likely infectious source. However, it has not been elucidated so far.

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Effect of copper sulphate on *Aedes aegypti* larvae in the laboratory

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Prospective controlled laboratory trials were conducted to determine the effect of copper sulphate ($CuSO_4.5H_2O$, 95.4%) on *Aedes aegypti* larvae using seven different concentrations, starting from 1.25 mg/L rising double strength up to 80.0 mg/L in accordance with WHO instructions on larval susceptibility test. These trials were undertaken in Health and Disease Control Unit, Mingaladon in October 2001 and included six replicates for each stage of larvae. The results showed that the larvae were highly susceptible to copper sulphate and its LC_{50} value for both stages was 2.25 mg/L and LC_{90} values were 10 mg/L and 15 mg/L for early and late stages respectively. Mosquito populations were found not to be significantly heterogeneous. Regarding residual effect, copper sulphate stock solutions (2,500 mg/L) of shelf-life day 1, 2, 3, 7, 10, 14, 21 and 28 were used and its effect against larvae normally persisted for about three weeks. This compound was found to be effective against the target species, harmless to human and environment, locally available and cheap. Therefore the role of copper sulphate was very promising to be used in the fields by its application in some minor water-storage containers, holding non-potable water such as altar flower vases, spiritual pots and bowls and ant-traps to suppress *Ae. aegypti* larvae in controlling dengue haemorrhagic fever effectively.

INTRODUCTION

Mosquitoes were commonly found in all types of flower containers except bronze vases in cemeteries in Florida, USA. In the laboratory test on *Ae. aegypti* larval development using plastic cups and bronze vases, 98% of larvae in the former completed development to adult stage whereas none in the latter survived beyond the second instar due to inhibition of larval development. It was suspected that copper, a main component of bronze vases, is the primary factor responsible for preventing mosquito development. In field surveys at cemeteries to find out mosquito prevalence in stone vases without liner, with aluminium liner and with copper liner, it was found that percentages of mosquito-positive vases were 60%, 50% and 11.7 % respectively. Percentage from vases with copper liner was significantly smaller ($p<0.01$). Various copper compounds are also used as fungicides and algicides [1, 2]. Copper sulphate {copper (II) sulphate or cupric sulphate}, one of the copper compounds, is easily available at local markets in Myanmar and also produced from Chemical Engineering Production Co-operative Society Ltd, Yangon (Khin Myo Win, personal communication, 2003). The present study was carried out with the objectives of [1] to determine the larvicidal effect of copper sulphate on *Ae. aegypti* (L.) larvae in 24 hours and [2] to assess its residual effect to be used in the control of dengue infection.

MATERIALS AND METHODS

The study design was a prospective controlled laboratory trial. The study period was from October, 2001 to January, 2003 and the larval collection area was Ward 3 Yanpye, Thaketa Township, Yangon, Myanmar.

Ae. aegypti larvae were collected randomly from at least twenty houses in the study area for the representative purposes. Copper sulphate (cupric sulphate pentahydrate, $CuSO_4.5H_2O$) and disposable plastic cups were purchased from local markets. Copper sulphate was chemically analyzed by using isometric method at Plant Protection Division, Myanmar Agriculture Service to determine the technical grade of cupric sulphate pentahydrate and the grade was found to be 95.4%.

Larval susceptibility tests were conducted, in accordance with WHO instructions [3], against early (first and second instars) and late (third and fourth instars) larval stages of *Ae. aegypti* using copper sulphate for the first time in Myanmar in the laboratory of Health and Disease Control Unit, Directorate of Medical Services, Mingaladon, Yangon. Seven concentrations starting from 1.25 mg/L rising double strength up to 80 mg/L were used for six replicates.

The larvae were first collected from domestic water containers (e.g.metal drums) in the study area using a clean plastic bucket (diameter 24.7 cm and height 25.0 cm) and then kept, for adaptation purposes, in a plastic tray (34.5 cm x 24.5 cm x 6.0 cm) containing rain water in the laboratory for one day before carrying out the test.

A stock solution was prepared by adding 1,048 mg of copper sulphate into 400 ml of rain water in a glass beaker (dia. 9.0 cm and height 11.8 cm) to get the concentration of 2,500 mg $CuSO_4.5H_2O$ per litre of water. 0.125 ml of stock solution was taken and added into first clean disposable cup (diameter 7.5 cm and height 11.5 cm) containing 224.88 ml of rain water and it was thoroughly stirred for 30 seconds with a glass rod. Then 0.25 ml of stock solution was added again into second cup containing 224.75 ml of rain water. Likewise, double the amount of stock solution was added till the seventh cup was completed. For control 225 ml of rain water was filled in a separate cup. At the same time each batch of active and vigorous 20 early stages (10 first and 10 second instars) together with natural food from their habitats was transferred from plastic tray to 8 small clean disposable cups (diameter 6.0 cm and height 4.8 cm) each containing 25 ml of rain water. Next each batch of 25 ml of rain water together with 20 larvae of early stages was introduced into seven test cups containing 225 ml of copper sulphate solution and into one control cup. The final concentrations of copper sulphate in test cups were 1.25 mg/L, 2.5 mg/L, 5.0 mg/L, 10.0 mg/L, 20.0 mg/L, 40.0 mg/L and 80.0 mg/L (Table 1). Moribund and dead larvae were counted as dead after 24 hours exposure. Mortality rates were recorded and plotted on a logarithmic-probability paper. The tests were done in six replicates. Similarly 20 larvae of late stages (10 third and 10 fourth instars) were tested in six replicates.

Table 1. Preparation of seven different concentrations of copper sulphate

No.	Initial rain water (ml)	Added stock solution (ml)	Added rain water (ml)	Resultant $CuSO_4$. $5H_2O$ concentration (mg/L)	Equivalent copper concentration (mg/L)
1	224.88	0.125	25	1.25	0.32
2	224.75	0.25	25	2.5	0.64
3	224.5	0.5	25	5	1.27
4	224	1	25	10	2.55
5	223	2	25	20	5.09
6	221	4	25	40	10.18
7	217	8	25	80	20.37

$CuSO_4$. $5H_2O$ concentration in copper sulphate stock solution is 2,500 mg/L.

For residual effect of copper sulphate, the stock solution (2,500 mg/L) was kept at room temperature for one, two, three, seven, ten, fourteen, twenty-one and twenty-eight days and then tested against ten larvae

(5 early and 5 late stages) in each concentration separately using three replicates. Then the mortality percentages were calculated after 24 hours exposure and they were compared. The temperature and relative humidity throughout the test period were 25 ± 7 ºC and 78 ± 8 % respectively. Data analyses were done using Epi Info Version 6.04 and S. Swaroop's statistical method for χ^2 test to determine correlation co-efficient and LC_{50} and LC_{90} values [4].

RESULTS

The test results showed that LC_{50} values of copper sulphate for both early and late stages was 2.25 mg/L and LC_{90} values for early and late stages were 10 mg and 15 mg/L respectively. The 95% confidence lower and upper limits were also described (Table 2, 3). There was a strong degree of correlation between dose and effect in both stages (r=0.66) but it was not statistically significant ($p>0.05$) due to small sample size of concentration of copper sulphate (n = 7). The χ^2 test for goodness of fit of the regression line showed that mosquito population were not found to be significantly heterogeneous in both early and late stages.

With regard to residual effect of copper sulphate it persisted for 21 days in first five concentrations. After 21 days it decreased gradually. Between 1st day and 28th day percentage reduction in larval mortality was from 9 % to 14 %. In the last two concentrations the residual effect did not reduce. Cent per cent mortality was found in the last concentration in all days up to 28th day (Fig.1).

DISCUSSION

Copper is widely used in cooking utensils, water distribution system, in making surgical instruments and intrauterine devices [5, 6]. In industry, it is used as an activator

Table 2. Fitting a regression line and testing the goodness of fit (Data on susceptibility of early larval stages of *Ae. aegypti* to copper sulphate)

No.	$CuSO_4.5H_2O$ concentration	Larvae dead/ tested	Observed mortality rate, (%) (adjusted)	Expected mortality rate (%) (from graph)	Observed minus expected rate	Contribution to χ^2
1	1.25	29/ 120	24	18	6	0.0244
2	2.5	60/ 120	50	56	-6	0.0146
3	5	85/ 20	71	78	-7	0.0286
4	10	106/ 120	88	89	-1	0.001
5	20	114/ 120	95	94	1	0.0018
6	40	118/ 120	98	97	1	0.0034
7	80	120/ 120	100 (99.38)	98.2	1.18	0.0079
8	Control	5/ 120	4		-	-
			Total			0.0817

LC_{50} = 2.25 mg/L,
95 % Confidence limit, lower = 1.95 mg/L
95 % Confidence limit, upper = 2.60 mg/L
LC_{90} = 10.0 mg/L,
95 % Confidence limit, lower = 7.92 mg/L
95 % Confidence limit, upper = 12.63 mg/L

Table 3. Fitting a regression line and testing the goodness of fit (Data on susceptibility of late larval stages of *Ae. aegypti* to copper sulphate)

No.	$CuSO_4.5H_2O$ concentration (mg/L)	Larvae dead/ tested	Observed mortality rate, (%) (adjusted)	Expected mortality rate (%) (from graph)	Observed minus expected rate	Contribution to χ^2
1	1.25	32/ 120	27	21	6	0.022
2	2.5	68/ 120	57	56	1	4E-04
3	5.0	93/ 120	78	76	2	0.002
4	10.0	104/ 120	87	87	0	0
5	20.0	106/ 120	88	92	-4	0.022
6	40.0	110/ 120	92	95.5	-4	0.029
7	80.0	120/ 120	100 (99.06)	97.2	1.9	0.013
8	Control	3/ 120	3	-	-	-
			Total			0.087

LC_{50} = 2.25 mg/L
95 % Confidence limit, lower = 1.86mg/L
95 % Confidence limit, upper = 2.72mg/L
LC_{90} = 15.0 mg/L
95 % Confidence limit, lower = 10.34mg/L
95 % Confidence limit, upper = 21.75 mg/L

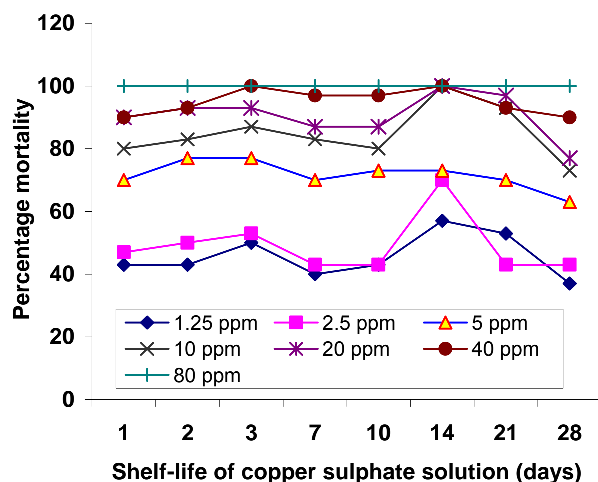

Fig.1. Residual effect of seven copper sulphate concentrations on *Ae. aegyti* larvae (both early and late stages)

in the froth flotation of sulphide ores, production of chromated copper arsenate wood preservative and others and in agriculture, it is used as a fungicide (Borde-aux mixture), pesticide and algicide, and nutritional supplements in animal feeds and growth promotors, as well as for disease control in livestock and poultry and as fertilizers. Latest approach to control of midge larvae in drinking water supplies is suppression of planktonic first stage larvae by using two disinfectants, chloramine and copper sulphate [7, 8].

Based on the results of a number of animal studies involving oral and intraperitoneal exposure to various copper compounds, it is generally agreed that copper and its salts are not animal carcinogens [9]. In prospective studies to determine associations between serum copper level generally greater than 1.25 mg/L and either total or breast cancer, there is no convincing evidence of dose-response trend. Moreover there has been no association between intake of copper and cancer in those few analytical epidemio-logical studies in which it has been investigated. There is also little convincing evidence that copper plays an aetiological role in the development of cancer in humans. The International Agency for Research on Cancer concluded in 1987 that there are no data on the carcinogenicity of copper 8-hydroxyquinoline in humans and insufficient data in animals. In connection with dermal exposure, it is non-toxic and though copper algicides are used in the treatment of water in swimming pools and reservoirs, there are no reports of toxicity from these application. No occupational studies were also found to indicate that copper exposure resulted in reproductive or developmental effects. On available data on human exposure worldwide but particularly in Europe and the Americas, there is a greater risk of health effect from deficiency of copper intake than from excess copper intake. A provisional tolerable daily intake (PTDI) of copper (Cu) from all sources was established as 0.5 mg/kg body weight [10]. As regard with content in water, copper should not exceed 3.0 ppm and sulphate ($SO^{=}_4$) should not exceed 250 ppm in natural or treated water [5]. WHO proposed that provisional guideline value of copper in drinking water was 2 mg / L [10].

In one of the laboratory tests the larvicidal properties of metallic copper against *Ae. albopictus* showed that development time from larva to adult was delayed and the resultant reduced weight of the adults probably influences both their fertility and flight ability [11]. The present study showed that the effect of copper sulphate on *Ae. aegypti* larvae was very satisfactory and correlation was strong but not statistically significant due to small sample size of copper sulphate concentrations. Its residual effect normally persisted for three weeks. Beyond three weeks it should be further tested in future. The optimal concentration was 15 mg/L and it was also the LC_{90} value for late larval stages as well as the dose not causing phytotoxicity in most plants and flowers in the vases (Htin-Zaw-Soe, unpublished data 2003).

Among the three categories of container type-breeding sites of *Ae. aegypti* - indoor and outdoor flower vases, plant pot saucers, spiritual pots and/or bowls (Nat-sin-ou) and ant-traps fall under the minor category.They are man-made and water is usually held in

them for a long time in the shade creating the well-breeding and well-producing sites of *Aedes* mosquitoes. Traditionally spiritual pots and bowls cannot be removed and eliminated. Among them some hold small messy plants like "Kanyut" (*Asparagus officinalis*) and "Myezar" or Bermuda grass (*Cynodon dactylon*) and householders were found to rarely empty them and change the plants, but frequently pour water on them. It was done like that due to their traditional belief which said that "The more the leaves of the plants sprout, the better is the household economy". In such condition copper sulphate should be used. To prevent larval breeding and emergence routine vector control method of changing water weekly should be done. But most of the people do not follow it though the method is simple and easy due to various reasons like state of being busy with daily works, scarcity of water and unwillingness to do so. Even if they follow they only change water in vases but do not thoroughly wash or scrub the roots or stems of the flowers or plants or inner wall of the vases. Therefore the eggs and larvae would be probably stuck to the roots and stems of the plants or to the inner wall of the vases thereby the remaining eggs and larvae may develop and finally the adults may emerge. Moreover, the flower vases and plant containers are usually placed indoors and mosquito-positive ones would make man-vector contact more easily than other outdoor containers. So attention must be paid to them though they are of minor category. In these conditions copper sulphate should be considered to be used. The dosage of copper sulphate is 15 mg/L every three weeks to have 90 % larval mortality. For a flower vase containing one litre of water it requires 15 mg of copper sulphate. The cost is only 0.03 kyat and it is very cheap. Metallic copper is also effective but its initial investment is larger than that of copper sulphate. Conventional larvicides like temephos (abate) are effective but expensive and imported from foreign countries at the large expense of foreign exchange. By using locally available copper sulphate, the country will prevent waste of foreign exchange.

It is concluded that the present study provides useful basic research data for appropriate technology for *Aedes* control and it could complement the present vector control programme. Therefore copper sulphate, a larvicide of being cheap, effective against target species, locally available, applicable and harmless to human and environment is recommended to be tested under field conditions and to be used in future in treatment especially of spiritual pots and bowls to control *Ae. aegypti* in our country where thousands of children under 15 years are stricken with dengue haemorrhagic fever annually.

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Factors influencing compliance with home-based self-care practices among people affected by leprosy with disability in Shwedaung and Thegone Townships

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A community-based descriptive study was undertaken in Shwedaung and Thegone Townships in Bago West Division in March 2005. The study aimed to investigate the level and factors of compliance with home-based-self-care practices among People Affected by Leprosy (PAL) with disability, and to provide basic information for further development of homes-based self-care package for prevention of disability. A total of 324 PAL were interviewed with a pre-tested questionnaire about the compliance by trained interviewers. Doing exercises of these PAL were observed and assessed by leprosy staff. Sixteen PAL were interviewed in depth. Generally, the study population had high compliance with exercises and low compliance with protection. Knowledge was associated with the level of compliance and the factors influencing compliance were discussed in detail.

INTRODUCTION

Globally, leprosy has been a public health problem for many years. However, with the changing characteristics of leprosy problem after declaration of leprosy, World Health Organization (WHO) more focus on prevention and/or worsening of disabilities (POD/POWD) for Patients Affected by Leprosy (PAL). Because of late treatment, although the disease was cured, some PAL had developed deformity [1]. Similar situation occurred in Myanmar since leprosy was eliminated in 2003. Up to now, in Myanmar, an extent of PAL with disability was one-fifth of the total cumulative released from treatment cases. So as to reduce the patients suffering from disability, a POD pilot project was conducted in Shwedaung and Thegone Townships in Bago West Division during 2002 to assess the relevance and feasibility of POD interventions [2]. This study showed that physical disabilities had improved to some extent, however, compliance with self-care routine was not optimal, which needs to identify the underlying factors of compliance for integration of information obtained into leprosy control programme of Myanmar.

Objectives

The study aimed to investigate level and factors of compliance with home-based self-care practices among PAL with disability in Shwedaung and Thegone Townships.

Specific objectives

(1) To determine level of compliance with home-based self-care practices among PAL with disability
(2) To identify factors influencing compliance with home-based self-care practices among PAL with disability
(3) To provide information input for further development of home-based-self-care package for POD

MATERIALS AND METHODS

A cross-sectional descriptive study was undertaken among PAL with grade I (i.e., anaesthesia present, but no visible deformity or damage) and grade II disability (i.e., visible deformity or damage present) during March 2005 in Shwedaung and Thegone Townships of Bago West Division. This was a continuation of POD pilot project, which was conducted in 2002, involving 395 registered PAL. During which, these PAL were taught by the leprosy vertical staff about home-based-self-care of their anaesthetic eyes, hands and feet [2]. The villages of the pilot project, where all original 395 registered PAL resided, were included in this present study. After getting consents, they were interviewed with a pre-tested questionnaire on demographic characteristics, practicing exercises and disability protection practices by trained interviewers. The trained leprosy staff observed disability's type of each PAL according to 1988-WHO disability grade with an impairment summary form, and assessed the correctness of doing eye-, hand- and foot-exercises. To triangulate the quantitative findings, in-depth interviews (IDI) were carried out with 16 PAL, 8 from each township. They were chosen by two dimensions (i) single disability (i.e., having disability on eyes, hands or feet) and multiple disabilities (i.e., having disability on at least eyes, hands and feet), and (ii) improved, worse or same impairment condition. They were asked about mainly on how and why they complied with instructions about home-based-self-care practices and exercises. Research team performed the IDIs. Quantitative data were analysed by SPSS 10 and field notes of IDIs were transcribed and then analysed manually.

RESULTS

Socio-demographic characteristics

Of 395 registered PAL, 324 (82%) were interviewed (Table 1).

Table 1. Distribution of the study population by socio-demographic characteristics of PAL (n = 324)

Characteristics	No. of PAL	%
Sex		
Male	221	68.2
Female	103	31.8
Age group (Years)		
<20	6	1.9
20 – 39	60	18.5
40 – 59	148	45.7
60+	110	34
Marital status		
Married	177	54.6
Single	82	25.3
Widowed	55	17
Separated /Divorced	10	3.1
Household member companionship		
Live with anybody else	302	93.2
Living alone	22	6.8
Level of education		
No formal schooling	98	30.2
Primary school	100	30.9
Middle school	107	33
High school	10	3.1
Collage/University	9	2.8
Type of occupation		
Private	110	34
Odd jobs	90	27.8
Dependant	89	27.5
Others	30	9.3
Government employee	5	1.5
Monthly family income (Kyats)		
2000 – 15000	123	38
15001 - 25000	79	24.4
25001 - 35000	49	15.1
35001 - 45000	15	4.6
45001 - 55000	15	4.6
>55001	6	1.9
No response	37	11.4

Male PAL were over 2 times the females, the majority were over 40 years (79.7%) and lived with family members or other relatives (93.2%), more than half were married, and about 64% had primary or secondary education. Nearly one-third was in private business. A little over one-fourth did odd jobs like selling seasonal fruits, working on farm, and driving trishaw. Twenty seven percent were dependants. Only a very few

were government employees. About three-fourths of their families had monthly income less than 35,000 kyats.

Clinical characteristics

Those with multiple disabilities were slightly higher than those with single disability (55.3% and 44.8% respectively). Disability in foot was the commonest- either foot alone or developed together with eyes and/or hands (Table 2).

Table 2. Distribution of the study population by disability pattern

Type of disability (n = 324)	No.	%
Eye only	6	1.9
Hand only	41	12.7
Foot only	98	30.2
Eye and hand only	1	0.3
Eye and foot only	7	2.2
Hand and foot only	142	43.8
Eye, hand and foot	29	8.9
Total	324	100

Level of compliance

Compliance with exercises

Any exercise practised two times a day for 10 days or once a day for 20 days per month was defined as low compliance and the score ranged between 0-20. Any exercise practised more than two times a day for 10 days or once a day for 20 days per month was defined as high compliance and the score ranged between 21 and 60.

Among the total study population, 198 (61%) had lagophthalmos (inability to close eyes fully), claw hands, foot drop or combinations and needed to do regular exercises of eyes, hands or feet. Nearly half (47.5%) of them was high in compliance with exercise for their respective disability condition, of which, a larger number was contributed by the eye exercises.

Compliance with personal protective practices

Compliance score on personal protective practices was developed on answers towards preventive measures of respective eyes, hands and feet (Table 3).

Table 3. Score development for personal protection

Site	Total score	Level	
		Low	High
Eye	0 – 9	0 – 5	6 – 9
Hand	0 – 5	0 – 3	4 – 5
Feet	0 – 9	0 – 5	6 – 9

The majority of them were low in level of compliance with protection practices but level of high compliance increased when it went from eyes through hands to feet.

Factors influencing level of compliance

Generally, while the majority had high compliance with exercises, they had low compliance with the protection of the affected body parts.

Compliance with exercises

Among the study population who needed to do regular exercises, generally, high compliance was found among males, under 40 years, divorcees, those who had no formal education or higher education, government employees, and those with low monthly income less than 15000 kyats. However, the differences between low and high compliance with exercises were not statistically significant for each socio-demographic characteristic.

Regarding knowledge of complications due to lack of care for affected body parts, those with some knowledge had high compliance with eye- and foot-exercises respectively (Table 4). There is highly significant difference between compliance levels for doing foot exercises.

Compliance with protection practices

Low compliance with protection practices was observed for all characteristics of PAL, but none of them were statistically significant. The majority of PAL with low level compliance had no knowledge about possible complications from lack of eye, hand and foot protection (Table 5). Highly statistically significant was found between the differences of levels of compliance.

Table 4. Level of compliance with exercises by knowledge of complications due to lack of eyes, hands and feet care

Knowledge	Level of compliance				Total	Chi square and p value
	Low		High			
	No.	%	No.	%		
Eyes						
No knowledge	7	38.9	11	61.1	18	0.37
Some knowledge	4	28.6	10	71.4	14	(p = 0.54)
Total	11	34.4	21	65.6	32	
Hands						
No knowledge	15	51.7	14	48.3	29	0.017
Some knowledge	67	50.4	66	49.6	133	(p = 0.89)
Total	82	50.6	80	49.4	162	
Feet						
No knowledge	6	100	0	0	6	7.17
Some knowledge	17	41.5	24	58.8	41	(p = 0.007)
Total	23	48.9	24	51.1	47	

Table 5. Level of compliance with protection practices by knowledge of complications due to lack of eyes, hands and feet care

Knowledge	Level of compliance				Total	Chi square and p value
	Low		High			
	No.	%	No.	%		
Eyes						p value for Fisher's Exact test = 0.0001
No knowledge	28	100	0	0	28	
Some knowledge	10	66.7	5	33.3	15	
Total	38	88.4	5	11.6	43	
Hands						
No knowledge	43	95.6	2	4.4	45	6.304
Some knowledge	134	79.8	34	20.2	168	(p = 0.012)
Total	177	83.1	36	16.9	213	
Feet						
No knowledge	47	87	7	13	54	15.314
Some knowledge	130	58.6	92	41.4	222	(p = 0.0001)
Total	177	64.1	99	35.9	276	

Knowledge was a key component for compliance as those who were knowledgeable had high compliance. This could be illustrated by some expressions. As they knew the benefit from doing exercises and were afraid of unpleasant consequences- physically and psychosocially, they performed exercises. They said:

"Doing eye exercises make better seeing. I think it's beneficial. I always do for better seeing"
(56 years, female, dependant, grade 2)

"Before exercise, there was foot drop. Now, if I didn't take care of my deformed feet, my feet would become worse and would be amputated"
(51 years, male, grow crops, grade 2)

"I felt ashamed as being a peculiar [disfigured] *person that unlike others"*
(22 years, female, odd jobs, grade 2)

Time factor played as a dual role for the compliance. While a night watcher who had the opportunity of getting enough time tended to do exercises frequently, one housewife said that she did not have time to do because of working the whole day for her family. Encouragement and assistance from the family also contributed to high compliance. On the contrary, those who did not get the family support were less likely to carry out the protection practices.

Despite the provision of health message on protection and protective devices by the health staff, some PAL pointed out that poor supervision of the staff was one of their reasons for low compliance. A very few said they saw no effect in preventing their affected body parts. One PAL with low compliance had negative feeling of doing exercise and stressed as follows:

"I hadn't done exercise regularly. Because, to say frankly, I don't think it would be better in spite of doing exercise. While the staff were demonstrating, I followed them, and I did it in front of them. Later, I didn't do it because I think there would be no progress"
(54 years, male, dependant, grade 2)

DISCUSSION

More or less, the study population had either single disability of Grade I or II and or multiple disabilities, where disability in foot was the commonest. PAL complied more

with exercises than with protection practices. Knowledge was associated with level of compliance where those who were aware of the benefit and consequences about protection had tended to follow the instructions for protection than those who were not. Time factor, family support and supervision also influenced the compliance. Low economic status, no free time due to daily activities, no interest by their family and poor supervision resulted in low compliance. Only a very few did not believe in preventive measures. However, PAL's characteristics were not associated with the level of compliance. This study reveals that for further implementation, we should consider to reinforce the PAL about the protection of their affected body parts, and to work together with the family, community and health workers for better home-based self-care.

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Bacteriological aspect of burns at Acute Burn Unit, Yangon General Hospital

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A cross-sectional descriptive study was carried out from June 2004 to April 2006 to determine the bacterial profile and antibiotic susceptibility pattern of burn isolates in patients admitted to Acute Burn Unit, Yangon General Hospital. Among 91 burn patients, 38 (41.8%) were culture-positive comprising pure isolates (27, 71.1%) and mixed isolates (11, 28.9%). The isolated bacterial pathogens were *Pseudomonas aeruginosa* (17/38, 44.8%), *Staphylococcus aureus* (14/38, 36.8%), *Klebsiella* species (3/38, 7.9%), *Proteus* species (3/38, 7.9%) and *Escherichia coli* (1/38, 2.6%). *Pseudomonas aeruginosa* isolates were sensitive to amikacin (100%), ciprofloxacin (94.1%) and gentamycin (82.3%). Augmentin and cefotaxime appeared to be most effective for *Staphylococcus aureus.* Majority of culture positive cases suffered from the burn wounds of buttock and leg. Burn injuries of more than 25% of total body surface area were found in 32 (84.2%) and those of less than 25% of total body surface area were found in 6 (15.8%) of 38 septic cases.

INTRODUCTION

Burn remains a major health problem throughout the world. Although survival after serious burn injuries has improved substantially during the past fifty years, infection of burn wound is still one of the leading causes of morbidity, mortality and long-term disability [1].

Following the initial period of shock, infection is the major complication in burns and it has been estimated that 75% of mortality associated with burns is related to infection. [2]. Extensive burns contribute to immunosuppression and this renders such patients prone to invasive bacterial infections. To ensure earlier and appropriate therapy in burn patients, most patients had been exposed to unnecessary antibiotics. Therefore, a continuous surveillance of microorganisms and regular up-date of their antibiotic resistance pattern is essential to maintain proper infection control programme respect to drugs choice for therapy [3].

As our country is on the way to develop as an industrialized nation, the incidence of burn injuries would be unavoidably increased as occupational hazard. Thus, the present study was carried out at Acute Burn Unit, Yangon General Hospital to detect common infectious organisms of burn and the current trends of antibiotic susceptibility pattern of them with the aim to provide updated information for management of burn injuries.

MATERIALS AND METHODS

The study design was hospital-based descriptive study. The wound swab samples were collected from a total of 91 burn

patients admitted to Acute Burn Unit, Yangon General Hospital from June 2004 to April 2006. They were inoculated into Stuart's transport media and transported to Bacteriology Research Division, Department of Medical Research (Lower Myanmar). After incubating overnight, they were inoculated onto Blood, Chocolate, Nutrient, Mannitol Salt, Ashdown and Mac Conkey agar and incubated again at 37°C overnight.

The suspected colonies were isolated and identified using Gram staining, biochemical testing and serological testing according to standard identification methods. Antibiotic susceptibility test was carried out using agar diffusion method.

RESULTS

Out of 91 cases, *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus, Klebsiella* & *E.coli* were isolated from 38 (41.8 %) cases and among them, 27 (71.1%) were single isolates and 11 (28.9%) were mixed isolates. The Gram-positive cocci were isolated from 14 (36.8%), cases and *Staph. aureus* was the highest isolated cocci in this study. The Gram-negative bacilli were isolated from 24 (63.2%) cases and the highest isolated bacillus was *Pseudomonas aeruginosa* (Table 1).

Table 1. The percentage of isolated Gram-positive cocci and Gram-negative bacilli

Isolated bacterial patho - gens (n=38)	Type of organisms	No. of isolates	Percentage of isolates
Gram- positive cocci	*Staphylococcus aureus*	14/38	36.8
Gram- negative bacilli	*Pseudomonas* spp.	17/38	44.8
	Klebsiella spp.	3/38	7.9
	Proteus spp.	3/38	7.9
	Escherichia coli	1/38	2.6

As shown in Table 2 the isolated Gram-positive cocci were highly susceptible to augmentin and cefotaxine and the isolated Gram-negative bacilli were highly susceptible to ciprofloxacin and amikacin.

It was observed that male patients were more suffered from burns compared to females and burn cases were more in 25 -35 years age group (Table 3).

Table 2. Antibiotic sensitivity pattern of isolated Gram-negative bacilli and Gram- positive cocci

No.	Type of anti-biotics	% of sensitive isolates		% of intermediate isolates		% of resistant isolates	
		*Gnb	**Gpc	*Gnb	**Gpc	*Gnb	**Gpc
1	Amikacin	91.7	71.4	8.3	-	-	28.6
2	Ciproflo-xacin	91.7	78.6	-	-	8.3	21.4
3	Genta-mycin	79.2	57.1	-	7.2	20.8	35.7
4	Septrin	79.2	57.1	-	-	20.8	42.9
5	Ampicillin	70.8	71.4	-	-	29.2	28.6
6	Augmen-tin	66.7	85.7	8.3	-	2.5	14.3
7	Cefota-xime	66.7	85.7	12.5	-	20.8	14.3
8	Ceftria-xine	62.5	64.3	20.8	14.3	16.7	21.4
9	Chloram-phenicol	l58.3	42.9	12.5	-	29.2	57.1
10	Penicillin	50.0	78.6		-	50.0	21.4
11	Cloxa-cillin	29.2	28.6	-	-	70.8	71.4

* Gnb = Gram-negative bacilli
** Gpc = Gram-positive cocci

Table 3. Age and sex distribution of burn cases with sepsis wounds

Age group (Year)	Sex		Total
	Male	Female	
> 5 - ≤ 15	2	-	2
>15 - ≤ 25	3	2	5
> 25 - ≤ 35	16	4	20
> 35 - ≤ 45	4	3	7
> 45 - 55	2	2	4
Total	27	11	38

DISCUSSION

Burns provide a suitable site for bacterial multiplication and more persistent richer sources of infection than surgical wounds, mainly because of the larger area involved and longer duration of patient stay in the hospital. In this study, culture was positive in 38/91 (41.8%) cases of burn unit. *Pseudomonas aeruginosa* was the

commonest isolated bacilli and this finding coincides with many previous reports by Agnihotri *et. al.* (2004) and Mc Mances *et. al*, [3,4]. Although *Staph. aureus* was the predominant organism in some reports [5,6,7,8], it was the second most common isolate in this study (Table1). Beta-haemolytic *streptococcus* was not isolated. Single bacteria isolation was more than mixed isolation and it was also in agreement with other studies [6, 8].

In this study, *Klebsiella* spp. accounted for 7.9% of total isolates. This is similar to Agnirotri *et. al.* (2004) report but other studies reported *Klebsiella* spp. as the leading pathogen in burn wound infections [3, 7 ,9].

For epidemiological and clinical purposes, the antibiotic sensitivity test of isolated bacteria is important. In this study, the predominant bacterial isolates were highly resistant to commonly available antibiotics. *Pseudomonas aeruginosa* was highly sensitive to amikacin (100%), ciprofloxacin (84.5%) and gentamycin (82.3%). *Staphylococcus aureus* was highly sensitive to augmentin and cefotaxime (85.7%).

The most common sites of infection in burn wounds were buttock (31.6%) and buttock and leg (26.3%) which may be due to pressure effect or contamination with excreta.

In conclusion, the early detection of isolates is also very important to prevent treatment failure. For the isolation and identification of bacteria, performing antibiotic sensitivity it could take 48 hours from receiving the specimen. This time period may be enough to allow a subclinical infection to become life threatening illness. Secondly, in burn wounds, because of the mixed infection, the potential virulence of one organism may affect another organisms growing alongside. Another factor adding to complication is multi-drug resistance of the organisms. Once MDR strain becomes established in the hospital environment this can persist for months. Therefore, careful microbiological surveillance and *in vitro* testing before the start of antibiotic therapy and restrictive antibiotic policy may be of great help in the prevention and treatment of MDR isolates in burn units thereby reducing overall infection related morbidity and mortality.

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Assessment of social acceptance and self image among Persons Affected by Leprosy in a community

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A community-based, cross-sectional study was conducted in Aunglan Township, Magwe Division in collaboration with Leprosy Control Programme in 2003-4. A total of 97 Persons Affected by Leprosy (PAL) was interviewed face to face with structured questionnaires after pre-test. A total of 11 In-depth Interviews (IDI) was undertaken among health care providers, friends and relatives of PALs. Findings were triangulated. Social acceptance was assessed by marital status, change of jobs, social contact with relatives friends and involvement in occasions in the community. Divorce rate was more in grade II PALs. Change of jobs was found to be 33% which were mainly due to disfigurement of the body. Family support seemed to be strong as most of GII cases were heads of household i.e.63.9%. Low self image was found. Acceptance by the family was observed as a key factor and parents are main persons close to PALs. Social acceptance by the community was noted to some extent. But 26.7% of PALs were never accompanied by friends. Since the social death of the PALs with disability represents the biggest challenge for rehabilitation, this baseline information will serve as a useful input for launching strategy in community-based rehabilitation program in Myanmar.

INTRODUCTION

In Myanmar, Leprosy Control Programme (LCP) has been launched to achieve the prevalence rate below 1/10,000 population [1]. In LCP, national strategies have been launched and one important issue is rehabilitation of disabled leprosy cases. In Myanmar, Community Based Rehabilitation (CBR) has been initiated mainly based on physical and health grounds. Concentration on medical care of people affected by leprosy with Multi Drug Therapy (MDT), surgery, etc. though vastly beneficial, has led to highly inadequate psycho-socio-economic rehabilitation in a holistic manner resulting in poor quality of life. Many studies on epidemiology, drug trials and operations research and health seeking behavior on leprosy have been documented but little is known about social factors which may intervene mainly in support and rehabilitation process [2-7]. Therefore, the attempt was made to explore the social acceptance and self image among grade 1 (GI) and grade 2 (GII) disabled Persons Affected by Leprosy (PAL). Based on this information, necessary measures will be taken by the family members and health care providers so that prevention of disability and rehabilitation can be achieved. The present project is an initial phase of three-phase project to explore social acceptance and self image of GI and II PALs in Aunglan Township, Magwe Division in late 2003 and early 2004.

MATERIALS AND METHODS

Study design

Community-based exploratory study design

Study methods

For quantitative, face-to-face interview with structured questionnaires was undertaken by trained interviewers. Semi-structured and open questions were included for assessing social acceptance and social image. For qualitative, Key Informant Interview (KII) with village female elders were done. For detailed information about social acceptance, self image, social stigma and needs, In-depth Interviews (IDI) to health care providers, relatives and friends of GI an II leprosy patients were performed by the investigators who have skill and experience in conducting qualitative research. All research tools were pre-tested.

Study area

Aunglan Township is located in Magwe Division, with a total population of 228,308. There are one 50 bedded hospital, two station hospitals and eight Rural Health Centers (RHC) for health care services. The township includes 92 village tracts with 240 villages. A total of 32 villages under three RHC areas namely (1) Aunglan Myoma Town proper (2) Kyaukpadaung (3) Pyalo and (4) Nyaungbinseik: were included in this study. The majority of villages are scattered and some areas are difficult to reach.

Study population

There were 150 registered grade I (GI) and grade II (GII) PALs at the time of study. Recruitment of the GI and GII cases and identification of their social surroundings was done by the township leprosy control programme. Out of registered 150 PALs with GI and GII disability, only 97 could be interviewed due to difficulties in finding cases that are living in the forest, moving out and hospitalized for reconstruction. For qualitative approach, detailed information about self image, social stigma and needs, were explored by 11 in-depth interview sessions with 4 health care providers, 3 relatives and 4 friends of GI and GII PALs.

Informed consent was provided to each interviewee and voluntary participation was made.

RESULTS

Age and sex

In the study group, male cases were found at 62.9% compared to female 37.1%. The age ranged from 10 to 84 years with the mean of 52.7 years.

Education

For educational status, majority of the respondents attained middle school (secondary) levels, few had university or graduates level. Illiterate persons accounted for 14%.

Marital status

Marital status was noted as an important indicator for assessing social acceptance. Out of 97 study patients, 90% were ever married and 10% were never married. Marital status by disability was observed that among GI cases, 66.6% were married, 16.7% were widow/widower and 16.7% were singles. For GII cases, 60.4% were found to be married 16.5% were widow/ widower, 15.4% were single and 7.7% were divorced.

Regarding frequency of marriage among the currently married group i.e. (49/59), 83% had first time marriage followed by 13.6% had second marriage and 3.4% had third time marriage.

Of divorced persons, not many reasons were explored but social problems between husband and wife mattered most i.e. 42.8%, some 42.8 % did not want to respond and only 14.4% were due to health.

Occupation

Among the study group in both sexes, 45.4% have been occupied. Of 44 PALs who were working at the time of survey, 31.8% owned farms and 45.5% were doing

odd jobs like mat weaving and fortune telling, and working in construction sites. Only 11.4% were paid workers in the farms compared to watchman at farm i.e. 2.3%.

Table 1. Study group by type of job

Type of employment	Type of job	Freq.	Percent
Self	Farmer	14	31.8
	Odd jobs	20	45.5
	Trishaw driving	2	4.5
	Lottery ticket selling	2	4.5
Employee	Paid worker	5	11.4
	Watchman	1	2.3
	Total	44	100

Of 19 cases who had changed from one job to another, 18 (94.7%) were GII and only 1 (5.3%) was GI cases. The only GI case said that he changed to a new job to earn more money. Of 18 GII cases, 33.3% had changed jobs because they had some problems with health. Few 11.1% of GII cases did not want to do anymore and another 11% had other reasons like having problem at work.

Table 2. Reasons for changing job by type of disability

Reason for changing	Disability (%) G I (n=1)	G II (n=18)	Total (n=19)
Need to get more earning	100	27.8	31.6
Health concern	0	33.3	31.6
Don't want to do	0	11.1	10.5
Problems at work*	0	11.0	10.5
Old age	0	5.6	5.3
No skill	0	5.6	5.3
No response	0	5.6	5.3
Total (n=19)	100	100	100

*Problems at work includes "problem with higher authority", "problem with other co-workers".

Main reasons for changing of work were desire to get more money, health reason and social problem. A total of 19 cases had changed from one job to another and 18 of them were GII cases. It was seen that dis-ability was the main issue for changing jobs.

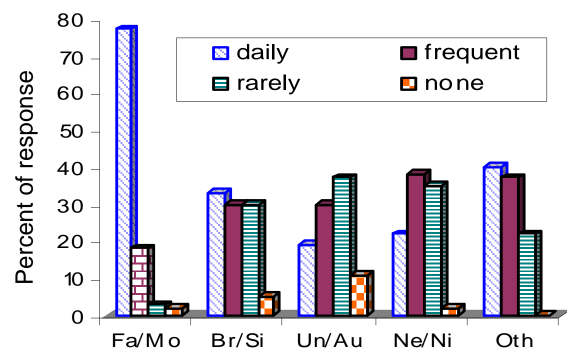
Status of PALs in the family

Identification of status of PALs in a family is assessing acceptance by the family. It was shown that majority of PALs 63.9% were heads of household.

Table 3. Role of PALs in the family

Head of household	Freq.	Percent
Respondent (PAL)	62	63.9
Spouse	16	16.5
Father	6	6.2
Mother	6	6.2
Brother / sister	4	4.1
Son / daughter	2	2.1
Grandfather	1	1.0
Total	97	100.0

Figure 1 indicates how frequent PALs contacted with their relatives by types. It was found that initial contact was made mainly by parents 77%, brothers and sisters 34% but 40% had contacts with other relatives who were not closely related. Among them parents made more contacts on a daily basis. Family members and relatives seemed to be close contacts for GI and GII cases in this study.



Fa/Mo =Father/Mother (29 cases)
Br/Si =Brother/Sister (78cases)
Un/Au = Uncle/Aunt (42cases)
Ne/Ni = Nephew/Niece (72 cases)
Oth = Others ((28 cases)

Fig. 1. Frequency of contact with relatives by PALs

Social contact with friends

The attitudes of friends on social contact with the PALs were studied. Most PALs (86.1%) felt that they are well accepted by the friends – always treated with good manner (93%), listened to what they said (90.7%), can talk to them friendly (89.5%) and can discuss with them always (50%). Some 10.5% have never discussed with friends and 26.7% said they did not accompany their friends.

Respondents perceived that although relatives and friends were socially accepting them as usual, they might be reluctant to chat and go out together.

Social occasions

Participation in outgoing social occasions was explored on religious affairs, festive occasions and other ceremonies like seeing patients, going to funeral and village affairs. They had been invited always for religious festivals and 44% did not go. For festive occasions, usually 54.6% of PALs were invited always but 5.2% said they were never invited. More than half i.e.53% participated in festive occasions. More than 50% of PALs had always been invited for social occasions like seeing relatives or friends while sick or death. Many of respondents who were invited did not involve with those occasions (44% to religious fairs, 47% to fun fairs and 32% ill/funerals). Activities for development of community in the village were participated by 67.5%.

DISCUSSION

Marital status was explored to check whether there is a link between disease and social issue. Remarriage, second or third times, was found and the main reason given was "PALs could not perform daily household chores" .This might be their bad feeling towards deformity. Changing from one job to another was found in GII PALs for health and social reasons and it was a challenge for social acceptance by the community. It was approved by seeing the number of changing jobs was 19 and of them 18 were GII cases. Instead of saying deformity they chose the word "**health concern**". Majority (63.9%) of PALs were heads of households. This was found to be positive factor of social image of PALs in the family. Social acceptance by the family was high. It coincided with the findings by Kyaw Myint *et al.* [8], in which the authors stated "***most patients were accepted either by their families or community".*** Leprosy and in turn, deformity and disability bring about deeper and fundamental changes in character, personality and attitude of people. These changes remain even after cured [9]. It needs to explore relationship among PALs, with their environment, family members, relatives, friends and community so that socio economic loss by the disease can be assessed. Parents and close relatives have more contacts than other relatives.

Without any reasons the majority of PALs did not go to festive or ceremonial occasions but they used to go to funerals or seeing sick persons. It showed they have low self image and confidence in this study. Although they showed low self confidence and image majority believed that they were helpful to family, friends and community. It was positive attitude towards social rehabilitation. Family is considered as the main support for disabled cases. Family members never devaluate the status of the cases and they pay more sympathy to the disabled ones. Therefore family members should be involved in training for self care and POD.

This study highlighted social acceptance and self image of PALs especially GII. Changing jobs indicates the insight feelings of PALs on social acceptance by the community. It is the high time to consider to implement social rehabilitation for PAL especially GII cases. Empowerment of PALs by proper counseling and self-care training to prevent complication and more damage to the affected sites are important. Reconstruction of deformity to gain self-confidence by PALs should be extended. Strategies for improving social acceptance and restoring self image were highly recommended to improve quality of life of PALs with deformity in the community.

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Establishment of a simple procedure to detect the yield of recombinant HBsAg protein expressed by transformed *Hansenula polymorpha* yeast cells (MCB)

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For large scale production of recombinant Hepatitis B vaccine, recombinant *Hansenula polymorpha* yeast cells containing the HBsAg -expressed gene (Master Cell Bank) was processed under cultivation in seed and main fermentors ,followed by cell disruption and purification by using concentrator, homogenizer, ultracentrifuge, column chromatography , and sterilization filtration to have a purified HBsAg bulk. The whole process takes about 2 months. In our study, a single colony of MCB strain was cultured in the simple culture tube with methanol feeding followed by glass bead disruption of recombinant yeast cells. The released HBsAg protein was determined by using the AUSZYME test kit. The identity and purity of recombinant HBsAg protein was confirmed by SDS PAGE and Western blot hybridization. The whole procedure took about one week. It also indicates the viability of recombinant *Hansenula polymorpha* yeast cells and stability of HBsAg - expressed gene in the MCB. This is a simple and rapid procedure to predict the yield of HBsAg protein expressed by recombinant *Hansenula polymorpha* yeast cells prior to the actual production process of recombinant HB vaccine in the Plant.

INTRODUCTION

In Myanmar, hepatitis B (HB) viral infection is one of the important national health problems with 10.36 % of HBsAg carrier rate [1] and 35-60 % of infection rate [2]. As there is no cure for chronic HB carriers, prevention is extremely important. The HB vaccine is the best protection against HB viral infection [3]. Safe and effective plasma-derived HB vaccine was successfully developed in Department of Medical Research (Lower Myanmar) in collaboration with WHO/ UNDP and locally distributed in 1997. In 2004, yeast-derived recombinant HB vaccine was successfully developed in the WHO GMP standard Plant, DMR (LM) with a capacity to produce 5 million doses annually under the EDCF Loan provided by the Republic of Korea. In production process of the recombinant HB vaccine, Master Cell Bank (i.e., recombinant HBsAg-expressed transformed *Hansenula polymorpha* yeast cells) was under cultivation in seed and main Fermentors, followed by cell harvesting, diafiltration, concentration, homogenization, pH precipitation, ultracentrifugation, gel chromatography and sterile filtration to have a final purified HBsAg bulk. This whole process takes about 8 weeks to identify the HBsAg protein in the product. Before starting the actual production process, it is necessary to know the productivity of HBsAg proteins expressed by MCB in time. In this study, MCB was cultured in our laboratory and HBsAg yield was detected by appropriate test methods. This study was

conducted with an aim to establish the test procedure to predict the HBsAg protein productivity expressed by MCB prior to the actual production process of recombinant HB vaccine.

MATERIALS AND METHODS

MCB strain (i.e lyophilized HBsAg-expressed recombinant *Hansenula polymerpha* yeast cells) was cultured in an appropriate media with addition of methanol to express HBsAg protein intracellularly. These cells were lysed by using physico-chemical procedures. The expressed HBsAg protein in media was determined by AUZYME ELISA test kit and confirmed by SDS PAGE and Western blot by hybrid-dization. The whole procedure was as follows:

Cell culturing and sampling

First, MCB was reconstituted with 1ml of autoclaved distilled water and 100 ul was cultured on 0.7% Yeast Nitrogen Base (YNB) with 2% glucose media culture plate at 30˚C for 48 hours. On the 3rd day, a single colony was inoculated into 10ml of Yeast extract Peptone (YP) broth media with 1% glucose in 50ml culture tube and incubated at 30˚C for 24 hours by using a shaking incubator with 250 rpm. On the next day, culture media broth was centrifuged at 3000 rpm for 15 minutes at room temperature(RT) followed by discarding supernatant and collecting pellets. These cells were resuspended in 20ml of YP media without glucose. After taking a sample volume of 1ml, culture tube containing these cells were incubated at 30˚C in shaking incubator with 250 rpm for 72 hours. During the incubation period, sampling of 1ml immediately followed by addition of calculated volume of methanol to make final concentration of 0.6% v/v at every 12 hours. (i.e. 0, 12, 24, 36, 48, 60 and 72 hours). Each sample was centrifuged 12000 rpm at RT for 2 minutes followed by discarding supernatant and collecting pellets.

Cell washing

Each sample was resuspended with same volume of autoclaved DW water followed by centrifugation at 4000 rpm for 10 minute at RT. Supernatant was discarded and pellet was collected, followed by resuspended with same volume of autoclaved distilled water. The above washing cycle was repeated 5 times for each sample to have complete cell washing.

Cell lysis

Each test tube containing pellets were weighed into 100 ug of cell mass (pellet), 50 ug of glass bead and 400 ul of buffer D solution (i.e. ratio of 2:1:8) were added followed by vortexing for 30 sec and keeping stand in ice berg for 30 sec. The above cell disruption cycle was repeated 10 times for each sample. Finally, all samples were centrifuged 12000 rpm for 15 min at RT, followed by discarding pellets and collecting supernatants which were kept at -20˚C till analyzed.

Determination of total protein and HBsAg contents

Each cultured sample was determined for total protein content and HBsAg content by using the Bradford Assay and the AUZYME test kit Monoclonal EIA (ABBOTT Lab) procedure respectively [4,5].

Identification of HBsAg protein by SDS-PAGE

Some cultured samples were subjected under SDS-PAGE by using SeeBlue Plus 2 protein standards and Silver X press staining Kit to detect the presence of HBsAg protein at the desired level of kDa.

Confirmation of HBsAg protein Southern blot hybridization

Finally, HBsAg protein containing SDS PAGE Gel was transferred onto the nylon membrane and subjected under Western blot hybridization procedure using specific antibodies to confirm the presence of HBsAg protein in the culture samples.

RESULTS

Total proteins and total HBsAg contents expressed by recombinant *Hansenula polymorpha* yeast cells at different times on cultivation with methanol feeding are shown in Table 1. Considerable amounts of total proteins and HBsAg were only expressed at 36 hours of cultivation, followed by increasing trend up to the 60 hours of cultivation. After that, the yields of total proteins and HBsAg became declined. The pattern of the HBsAg protein expression is clearly illustrated in Fig.1. It shows the productive capability of recombinant *Hansenula polymorpha* yeast cells in MCB to express HBsAg protein .

Table 1. Determination of total proteins and HBsAg contents in different times of cultivation with methanol feeding

Sample No.	Time of cultivation	Total volume(ul)	Total proteins(ng)	Total HBsAg (ng)
1	0 hr	40	44	2.60
2	12 hr	32	36	2.24
3	24 hr	24	15	2.58
4	36 hr	78	34	13.2
5	48 hr	80	87	79.8
6	62 hr	120	108	82.8
7	72 hr	188	39	12.2

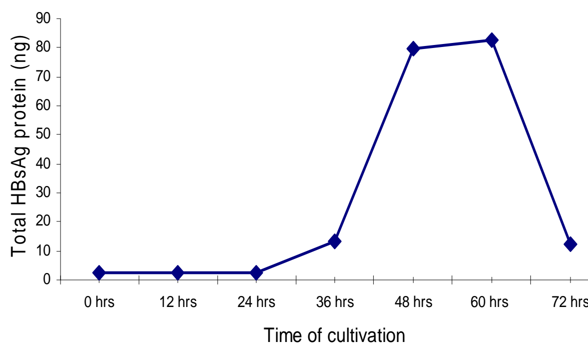


Fig.1. Pattern of the HBsAg protein productivity expressed by *Hansenula polymorpha* yeast cells in cultivation with methanol feeding

SDS-PAGE determination of the HBsAg proteins samples at various time intervals of cultivation was illustrated in Fig. 2. Since the volumes and amounts of total protein and HBsAg expressed at 0, 12, and 24 hours of cultivation were too low, these samples were insufficient to apply in SDS-PAGE determination. However, distinct bands were identified in lane 2, 3, 4 and 5 at the level of 24 kDa which is the theoretical molecular weight consistent with that of HBsAg protein, thus representing the HBsAg proteins of 36, 48, 60 and 72 hours of cultivation respectively. It confirms the presence of the HBsAg protein in these samples expressed by MCB.

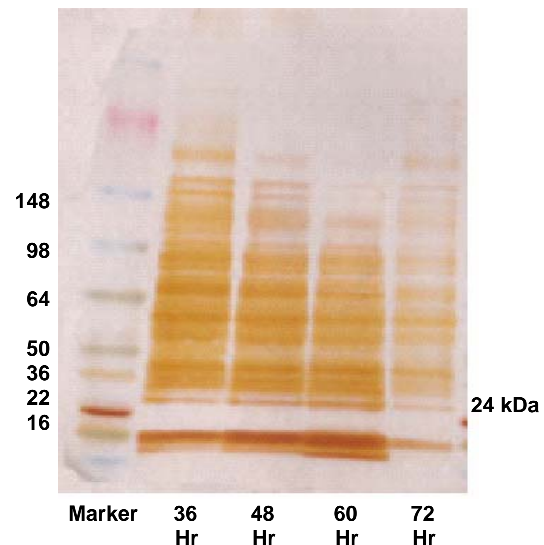


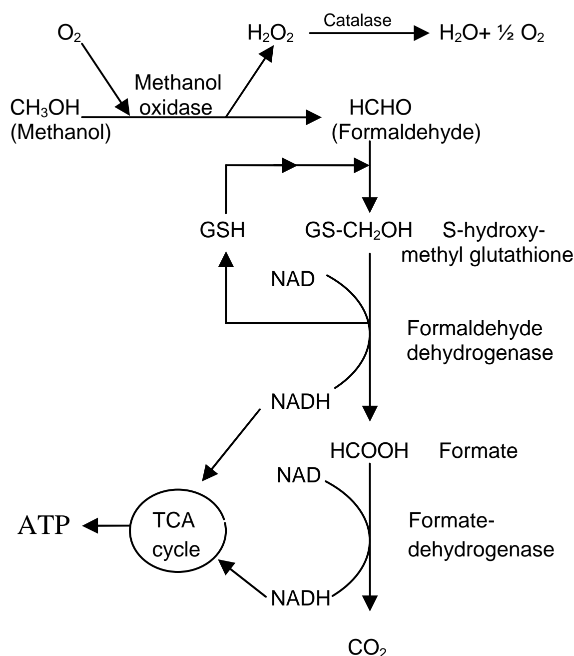
Fig. 2. SDS-PAGE determination of HBsAg protein in various cultured samples at different time intervals.

After transfer of HBsAg protein bands containing SDS-PAGE gel onto the nylon membrane, these bands were identified by using specific probes for HBsAg protein in Western blot hybridization technique. It was found that clear and distinct HBsAg bands were observed in samples of cultivation for different time intervals thus directly confirming the HBsAg productivity of recombinant *Hansenula polymorpha* yeast cells (Figure not showing).

DISCUSSION

For production of recombinant HB vaccine in the Hepatitis B Vaccine Plant, MCB containing HBsAg expressed-*Hansenula polymorpha* yeast cells (MCB) provided by

CJ Corporation, Republic of Korea has been used as a starting material, followed by fermentation and purification processes by using sophisticated machines and complicated procedures to obtain a purified HBsAg bulk. It is well documented that *Hansenula polymorpha* is a methylotrophic yeast and is a very useful system for manufacturing recombinant proteins [6, 7]. Because of the presence of Methanol oxidase (MOX) promoter gene in chromosomal DNA of transformed *Hansenula polymorpha* yeast cell containing the HBsAg structural gene (MCB), it is capable of metabolizing methanol as its sole carbon and energy source.



A schematic presentation of methanol metabolism in transformed *Hansenula polymorpha* yeast cell

The first step in the metabolism of methanol is the oxidation of methanol to formaldehyde using molecular oxygen by the enzyme methanol oxidase (MOX), expressed by the MOX promoter gene present in the HBsAg expression vector incorporated in the chromosomal DNA of *Hansenula polymorpha* yeast cells. This reaction also generates hydrogen peroxide which is very toxic to the host cell and is degraded by catalase. Formaldehyde first condenses non-enzymatically with reduced glutathione (GSH) to form 5-hydroxy methyl glutathione (GS-CH_2OH). This product is hydrolyzed to formate and oxidized to carbondioxide by formaldehyde dehydrogenase and formate dehydrogenase respectively. This process generates NADH molecules which are required for formation of high energy rich compound, Adenosin Triphosphate (ATP) through the Tricarboxylic acid cycle used for biosynthetic purpose.

Therefore, in this study methanol was added as an inducer for MOX promoter gene incorporated in the chromosal DNA of these cells to express the desired protein during cultivation period. Since the expression is intracellular, lysis of these cells was required. For this purpose, vortexing with glass beads was performed for rupturing of cell wall in our study.

On studying the productive capability (yield) of HBsAg protein expressed by MCB in our laboratory, it was found that the MCB expressed the detectable level of HBsAg protein from the time of beginning, throughout the cultivation period, and up to 72 hours, obtaining the maximum expression at 60 hours of cultivation from which HBsAg expression quantity became declined. The expressed HBsAg proteins were identified in SDS-PAGE and confirmed by Western blot analysis.

The procedures of cell cultivation, cell disruption and HBsAg protein identification in this study were very simple, and only routinely used laboratory instruments and equipment were required to obtain the desired and expected protein. In addition, the whole procedure took about one week whereas the actual production process lasts about two months to identify the HBsAg protein expressed by the starting MCB in the intermediate product.

Recently, viability of the HBsAg protein-expressed recombinant *Hansenula polymorpha* yeast cells and stability of the

recombinant HBsAg expression vector in genomic DNA of *Hansenula polymorpha* yeast cells in MCB were already studied and reported [8, 9]. The results of our findings directly confirmed that the yield expressed by MCB was found to be the expected HBsAg protein. It also indirectly confirmed that lyophilized recombinant *Hansenula polymorpha* yeast cells in MCB stored for a certain period were found to be viable and the HBsAg protein expressed gene incorporated in chromosomal DNA was also tested to be stable in MCB. Therefore, this simple and rapid procedure could be used to predict the yield of HBsAg protein expressed by recombinant *Hansenula polymorpha* yeast cells of MCB prior to the actual production process of recombinant HB vaccine in the Plant.

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Production of Russell's viper (*Daboia russelii siamensis*) antivenom in laying hens

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Traditional antivenoms raised in horses carry complement-mediated side effects, serum sickness and occasional anaphylactic shock. In order to circumvent these side effects and to supplement antivenom production, it was attempted to induce Russell's viper (*Doboia russelii siamensis*) antivenom in laying hens. Three injections of Russell's viper venom, a total of (500µg per hen) given at 4 week intervals yielded 1.85 gm specific chicken immunoglobulin IgY per month which is equivalent to total IgG obtained from 8 rabbits or two goats per month. The IgY antibody extracted from egg yolk with polyethylene glycol 6000 could withstand $2LD_{50}$ dose of the immunizing venom (ED_{50} =29.7µl/mouse). Antibody IgY level in egg yolk was determined by indirect enzyme immunoassay method and specificity of the antibody was checked by immunodiffusion. The antibody could be detected as early as 2 weeks after the first immunization and peak antibody levels were maintained up to 20 weeks before declining to a low level. The major advantages of the avian antivenom are the eggs from immunized hens provide a continual source of antibody, it is inexpensive to keep hens as laboratory animals and require only 3 injections using minute amounts of the venom (500µg/hen). The study highlighted that antivenom of interest could be raised by using this simple technique and the avian Russell's viper antivenom could be used for treating Russell's viper bite cases as well as to supplement antivenom production of the country.

INTRODUCTION

Russell's viper bite is endemic in Myanmar and the trend of snakebite in the country is on an increase based on hospital returns collected by the Department of Health Planning. The average annual poisonous snakebite of the whole country (1998-2005) are 8107 (6529-9600) with a case fatality rate of 7.43% (4.93-8.82%) [1]. In order to meet the requirement of increased demand of Russell's viper anti-venom, ways and means of production or supplementing antivenom production are sought for. The mainstay of management of snakebite is administration of specific antidote (antivenom) which is usually raised in horses. Administration of equine antivenom carries clinical side effects such as anticomplementary reactions, serum sickness and sometimes anaphylactic shock. In order to circumvent these side effects, production of antivenom in laying hens was attempted. The yolk of eggs laid by immunized hens has been recognized as an excellent source of polyclonal antibody [2, 3, 4]. Avian antivenoms raised against a variety of venoms have been reported [5,6,7].

MATERIALS AND METHODS

Immunisation

Four 16-week-old ISA Brown (local name CP Brown) hens were each immunized with 100µg of Russell's viper (*Daboia russelii siamensis*) venom (Myanmar Pharmaceutical Factory, MPF) in complete Freund's

adjuvant (CFA) (Gibco) on day 0 subcutaneously at six sites (both sides of the abdomen, both breasts and in both wings). The hens were further immunized with 200 µg of the same venom in incomplete Freund's adjuvant (IFA) (Gibco) on days 28 and 56.

This study was carried out in the Lane gone village of Shwepyithar Township, Yangon division from March to August 2006 which lasted for 5 ½ months. Daily collection of eggs began 2 weeks after the first immunization until the end of the study. Eggs were labeled and kept in a sand pot embedded in the ground with daily watering of the sand before being dispatched to the Department of Medical Research (LM) every weekend. They were stored at 4°C until analysed.

Collection and purification of antivenom

Chicken immunoglobulin IgY (IgG) was extracted according to the method of Polson *et al*. [8]. The broken yolks were blended with 7 volumes of egg extraction buffer (0.01 M PBS, pH 7.5) to improve antibody yield and polyethylene glycol 6000 (PEG) was added to a concentration of 3.5%. The mixture was incubated for 30 minutes with stirring and then centrifuged at 10,000 rpm for 30 minutes. After centrifugation, the precipitate was discarded. The supernatant was decanted, filtered through gauze to remove the lipid layer and PEG was added to it to a final concentration of 12% and stirred for 30 minutes, followed by 30min of centrifugation at 10,000 rpm. The supernatant was then discarded and the pellet dissolved in 0.01 M PBS, pH 7.5 and centrifuged twice to remove the PEG. The crude IgY pellet was dissolved in the original volume of egg extraction buffer 0.1 M PBS pH 7.5 and stored at 4°C until analysed.

Characterisation of immunogen (venom)

Determination of Median Lethal Dose

The lethal toxicity of the Russell's viper venom (*Daboia russelii siamensis*) was assessed by intravenous injection of 0.2 ml of the venom in physiological saline into tail vein of 18-20 gm male ICR (Institute of Cancer Research) mice. Six mice were used for each venom dose. For control, six mice were injected with normal saline. Death of the animals following 24 hours after injection was noted. The LD_{50} (intravenous) of the venom was calculated by probit analysis [9].

Determination of venom neutralising efficacy of chicken IgY antibody (antivenom)

Neutralisation of lethal activity of the venom by the antivenom (ED_{50}) was performed according to the WHO recommended standard test of neutralizing activity [10]. Peak purified IgY antibody samples collected at 10, 12, 18 and 20 weeks after the first injection were tested for venom neutralizing activity. Briefly 100µl of a fixed amount ($2LD_{50}$=9.6 µg) of Russell's viper venom was incubated with an equal volume of varying amount of chicken IgY antivenom for 30 minutes at 37°C and injected into ICR mice and death of the animals within 24 hours following the injections were recorded. The ED_{50} was calculated according to the recommended WHO method [10].

Immunodiffusion

Antigen-antibody reaction was performed in 1.5% agarose gel plate using Ouchterlony's double diffusion methods [11]. IgY antibody (antivenom) samples collected at 10, 12, 18 and 20 weeks after the first injection and 1:4 dilution of Russell's viper antivenom manufactured by Myanmar Pharmaceutical Factory (batch C 98011, expiry 4/2001) were put up against 1mg/ml of the immunizing Russell's viper venom in a moist chamber at room temperature for 24 hours. The plate was washed with physiological saline and stained with 0.025% w/v Coomassie brilliant blue R-250.

Determination of antibody levels in egg yolk of immunized hens

IgY antibody levels in egg yolks of the immunized hens were determined by

indirect enzyme linked immuno assay (EIA) [12]. In brief, 96-well microtitre plate (Nunc-Immuno U) was coated with 100µl of 2 µg/ml of Russell's viper venom in 0.01 M coating buffer pH 9.6 overnight at 4°C. The plate was then washed with PBS-Tween, remaining unbound free binding sites were blocked with 3% BSA-0.01 M PBS for one hour at 37˚C. After washing with PBS-Tween, 100µl of samples (chicken IgY) were added to the wells and incubated at 37˚C for one hour. The plate was washed again with PBS-Tween 20 and 100µl of 1:10,000 dilution of alkaline phosphatase conjugated rabbit anti-chicken IgY (IgG) (Sigma) was added and incubated for one hour at 37˚C. The plate was washed with PBS-Tween 20 and the final reaction revealed by adding 100 µl of substrate made up of 5 mg/ml PNP (P- Nitrophenyl Phosphate) in 10% diethanolamine buffer pH 9.8. The reaction was stopped after 30 minute incubation at room temperature by adding 50µl/well of 3M NaOH to the wells. The colour developed was read at 405 nm wavelength in EIA reader (Humareader).

Determination of the IgY concentration and yield per ml of yolk.

The absorbance of 1:20 diluted purified IgY in 0.01 M PBS, pH 7.5 was measured at 280 nm in 10 mm path length quartz using PBS as reference. The IgY concentration (mg of IgY per ml) was calculated by using the formula where the absorbance of 1mg/ml IgY solution at 280 nm is 1.4 [2].

Determination of specific IgY

The specific IgY concentration of the crude IgY was determined by immuno-precipitation method [2]. To 0.5 ml of the crude IgY solution, 1 ml of a 0.01% antigen (Russell's viper venom) in 0.01M PBS pH 7.5 was added and incubated overnight at 37°C. The supernatant was separated by centrifugation at 3,000 rpm for 30 min and its absorbance was measured at 280 nm. The absorbance of the crude IgY was also measured.

Determination of total protein content of IgY

The protein content of the IgY solution was determined by Lowry method [13] using a standard curve generated with bovine serum albumin (Sigma).

Polyacrylamide gel electrophoresis (PAGE)

The purity and molecular weight of the protein and its subunits were determined by performing SDS-PAGE as described by Laemmli [14] using 10% separating and 4% stacking gels. The samples were dissolved in reducing SDS buffer and heated at 95˚C for 5 min. Twenty microlitres of samples were loaded to the troughs. Electrophoresis was carried out at 200 V for 45 min. using Mini Protean ll electrophoresis (Bio-Rad). The protein bands were stained with 0.1% Commassie brilliant blue R-250 and destained with 20% methanol in 10% acetic acid until a clear background was obtained.

RESULTS

Purification of egg yolk IgY antibody

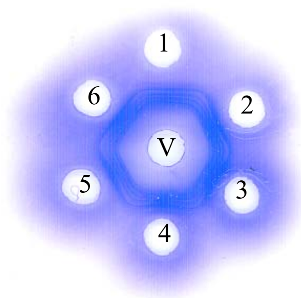
On average, laying hens (n=4) lay 22 eggs per month. The average weight of an egg is 52.38 gm ± 3.62 (1SD) (range 43.29-59 gm) and mean volume of the yolk is 13.94 ml ±1.97 (10-17.5 ml). The IgY content of the yolk is 6.05 mg/ml ±0.514 (4.82-6.87 mg/ml) or 84.38 mg IgY per egg. Purity of IgY is 87.35% (86.04-89.97%). IgY yield per month is 1.85 gm and specific IgY is 38.16% (26.12-48%). IgY protein content (Lowry method) is 6.79 mg/ml ±0.40 (5.6-6.98 mg/ml).

Immunodiffusion

The egg yolk (IgY) antivenom collected at 10, 12, 18 and 20 weeks after the first injection and the MPF antivenom showed six precipiting lines with the immunizing Russell's viper venom (Fig. 1).

SDS PAGE

SDS PAGE electrophoresis of the chicken IgY sample shows 4 major protein bands, 180 kD, 66 kD,50 kD and 31 kD (Fig. 2).



1 ~ 2 = MPF antivenom (1:4 dilution)
3 ~ 6 = peak chicken IgY samples
V = Russell's viper venom (1 mg/ml)

Fig.1. Immunodiffusion between Russell's viper venom and chicken IgY and MPF antivenoms

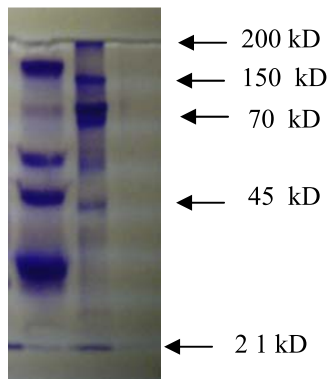


Fig. 2. SDS-PAGE electrophoresis of purified chicken IgY
Left lane = purified chicken IgY
Right lane = molecular weight markers

Development of egg yolk IgY antibodies in immunized laying hens

The IgY antibody (antivenom) was first detected in the egg yolk of the immunized hens two weeks after the first injection, reaching its peak at 2 weeks after the third immunization and maintained at its peak up to 20 weeks after the first immunization before declining to low level (Fig. 3). A transient dip in antibody level was observed at 12th week after the first immunization.

Venom neutralizing efficacy of IgY antibody (antivenom)

The $LD_{50i.v}$ of the immunizing Russell's viper venom is 4.8 µg/mouse (95% confidence levels 4.32-5.28 µg/mouse). The four peak IgY antibody (antivenom) samples

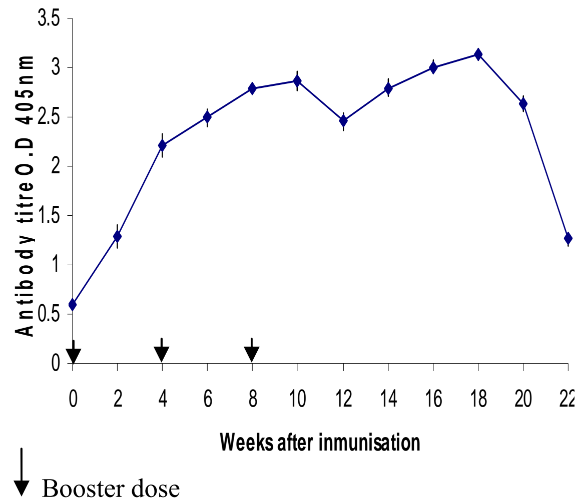


Fig. 3. Antibody titers in chicken egg yolk purified by PEG (Polyethylene Glycol 6000) after immunisation with 100 µg and 200µg of Russell's viper venom with adjuvant. The arrows indicate time of immunisation. Each point represents mean of three determinations. Bars represent standard deviations.

collected at 10,12,18 and 20 weeks after the first immunization could withstand the challenge of $2LD_{50}$ i.v. of the immunizing Russell's viper venom. The ED_{50} of the antivenoms (IgY) were 24.71 µl/mouse, 37.7 µl/mouse, 24.07 µl/mouse and 32.3 µl/mouse respectively. Mean ED_{50} was 29.69 µl/mouse (95% confidence levels 23.18-36.2 µl/mouse). One mililitre of IgY antibody (6.05 mg/ml) could neutralize 0.334 mg of the Russell's viper venom.

DISCUSSION

The study demonstrated that relatively pure Russell's viper antivenom could be obtained from the yolk of immunized laying hens. The purified IgY antibody (antivenom) could be extracted from egg yolk with polyethylene glycol. It is venom specific and has a purity of 87%. The peak IgY antibodies could withstand 2 LD_{50} of the immunizing venom. A transient drop in the antibody level at 12 weeks after the first immunization was due to ill health of the hens secondary to change in environmental housing conditions which

lasted for 4 weeks. IgY antibody could be detected in the yolk up to 100 days after three immunizations. In order to maintain peak antibody levels, subsequent boostings with the antigen are required.

In this study we used minute amount of native antigen and obtained 1.86 gm of IgY antibody per month (22 eggs/month) which is equivalent to a yield of total IgG antibody from 8 rabbits (250 gm/50 ml/month/rabbit) and that of 2 goats (1 gm/200 ml/ month/ goat) per month [15].

Earlier studies on efficacy of avian antivenoms (*Crotalus atrox* and *Trimerusurus flavoviridis*) following use of large ascending doses (mg) of the modified respective venoms and hyper immunisation schedule indicated that the antivenoms were 2 and 6.3 times more potent than the respective commercial equine antivenoms [6]. In the present study the efficacy of the IgY antivenom was found to be two times less potent than the MPF Russell's viper antivenom. It is likely that use of minute amounts of crude Russell's viper venom (500 ug per hen) with short immunization schedule (three injections) could have attributed to it. Ideally in order to have antivenom capable of neutralizing all toxic components of the venom, unmodified venom should be used rather than relying on detoxification of venom which could lead to reduction or complete loss of important antigen [16]. Formal toxoiding of Russell's viper venom resulting in reduction in biological activities of the venom has been reported [17]. For this reason, we used unmodified venom for immunization which limits use of high doses of the venom unless it is detoxified. However, the efficacy of IgY antivenom could be increased by either concentrating the final product or use of a slightly higher dose of venom or modified venom with hyper immunisation carried out in earlier cases [6].

Egg laying capacity of the laying hens declines after 100 days with slow recovery of capacity. For cost effectiveness of the study, the experiment was terminated at 100 days after the first immunization which coincides with the decline in antibody level. However it could be boosted in order to maintain the antibody levels.

The advantages of avian antivenom are (a) the eggs from immunized hens provide a continuous daily source of antivenom and avoids animal bleeding, (b) IgY antibody does not fix mammalian complement thus avoiding complement mediated side effects, (c) keeping chickens as laboratory animals is inexpensive (requires almost the same procedure as keeping other laboratory animals), (d) IgY extracted from egg yolk is pure and venom specific and (e) antibody productivity of an egg laying hen is much greater than that of a similar sized mammal, (f) purified chicken antibodies have higher bio-activity than those raised in horses (g) it minimizes administration of large volumes of foreign protein to the patients and (h) it is safer and more economical to produce compared to horse antivenom. The use of chickens for production of antibodies is attractive from an ethical viewpoint with respect to Russell and Burch's principles of the three Rs – the principle of replacing, reducing and/or refining the use of laboratory animals when possible [18].

In conclusion the study demonstrated that pure potent IgY antibody (avian antivenom) extracted from egg yolk could be used for treating Russell's viper bite cases and also to supplement the current antivenom production of the country. Moreover, this simple technology could be used for production of important and common antivenoms and has been used as successful agents for passive and protective immunization against gastrointestinal pathogens in humans and animals [15].

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Cost for birth delivery of rural mothers from Kyaunkpadaung Township, Myanmar

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The cross-sectional study was conducted during June 2005 to determine the factors influencing birth delivery utilization and to estimate cost for birth delivery of rural mothers from Kyaunkpadaung Township. Randomly chosen 750 mothers who had delivered last two years were interviewed with a pre-tested questionnaire. About 60% of mothers delivered by midwives followed by traditional birth attendants (TBAs) and auxiliary midwife (AMWs), and home deliveries were the highest (90.7%). Common reasons were financial problem, social circumstances, experiences and a local custom of giving births at home. Basically, the majority had spent a maximum of 50,000 Kyats, which ranged from 150 to 221,000 Kyats for delivery. If they were absent from work during delivery period, it increased to 794,000 Kyats. Large amount of basic expenses for delivery was contributed by consultation fee of general private practitioner/obstetrician and gynaecologist (GP/OG) and high cost of hospitalization.

INTRODUCTION

In rural areas of Myanmar, home deliveries are the highest (80%). Of which, 44.4% delivered by nurse/midwife, 38.1% by TBA and 5.5% by other unskilled personnel such as relatives, neighbours or herself. Only 11.5% were delivered by doctors (Population Department and UNFPA 1999). The same pattern was found in Kyaukpadaung Township in 2002 - home deliveries by midwives were 41.8%, and by AMW and TBA collectively was 39% (Department of Health 2004). The reverse was observed in Kyaunkpadaung during 2003 and 2004, the percentages of home deliveries by AMW/TBA were slightly higher than by midwives - 48.3% and 42% in 2003, and 50.2% and 47% in 2004. This indicates that skilled birth attendant – midwives were not fully utilized in rural areas. However, factors influencing this situation are not known broadly. At the same time, there was lacking in studies on cost of birth delivery in Myanmar. This study thus attempted to determine the factors influencing birth delivery utilization and cost of birth delivery. The objective is to determine cost of birth delivery and factors influencing birth delivery utilization of rural mothers from Kyaunkpadaung Township.

MATERIALS AND METHODS

A cross-sectional study design was carried out during June 2005 in Kyaunkpadaung Township. The study included 30 health centres, namely two station hospitals, seven rural health centres and a sample of 21 rural health subcentres. Health centre-based village tracts were grouped into 30 clusters of village tracts - one village tract per health centre was chosen randomly.

Regardless of the type of birth attendant, a total of 750 mothers who delivered the youngest child alive during last two years, whether they were pregnant at the time of survey or not, were chosen randomly. Those who had abortion were excluded. Twenty-five mothers per cluster were selected at random from a list of eligible mothers. If the selected cluster had not enough mothers, the adjacent cluster was taken into consideration. To obtain 750 mothers, we interviewed more than 25 mothers for some clusters since four health centres were dropped due to difficulties in transportation because of heavy rain during the main survey period. The trained interviewers asked the selected mothers with a pre-tested semi-structured questionnaire at a private environment.

RESULTS

Identification of mothers

Mean age of 750 mothers was 29.6 years with a range of 16 to 48 years. The majority of them had lower education or no schooling (73.2%) and only 3.2% were graduates. Most of the mothers' family members belonged to a lower social group* (63.3%), and the majority of mothers were working mothers (70.4%). The majority of mothers had a few children (75.5% had maximum 3 children and 24.5% had more than 4 children).

Delivery: birth attendant and place of delivery

Birth attendant was defined as a main person who pulled the baby regardless of the qualification and delivery skill. Most mothers gave births by skilled birth attendants-midwife (59.5%), hospital staff (7.6%) and GP/OG (0.8%) (Table1). Some mothers delivered by unskilled birth attendants ⁻TBA (19.9%) and AMW (9.1%). While 16 mothers were delivered by their female relatives, 8 mothers reported that they delivered by themselves. None of them had training for delivery; however, they had practical experiences in giving births. The majority (90.8%) gave births at their homes and about 8% at hospital (Table 1).

Cost for birth delivery of the youngest child

We considered two types of cost regarding delivery – basic expenses and an additional cost in terms of earning loss of the mother and her family for not working during delivery period.

Basic expenses

Basically, the majority (94%) had spent a maximum of 50,000 Kyats with a range from 150 to 221,000 Kyats. Generally, they used them for preparation for delivery such as transportation, firewood, clothes and medicines. Additionally, they had to spend for consultation fee and it varied depending on the situation. If they delivered by GP/OG, they were charged at least 5,000 Kyats. The fees ranged between 3,000 and 5,000 Kyats if they deliverd by midwives and it ranged from 1,500 to 3,500 Kyats by AMWs. TBAs received 2,000 Kyats the most. Sometimes,instead of money, mothers gave fabrics, food or helped with the house work.

Although midwife's fee was slighlty higher than of AMW and TBA, most gave births by midwives, followed by TBAs and AMWs (59.5%, 19.9%, 9.1% respectively) (Table 1). Of which, the largest number of mothers (63.3%) was from the lower group and the majority were home deliveries (441/ 476 mothers). When probing, nearly all mothers said the residential birth attendants-midwives, TBAs and AMWs were easily accessible for home deliveries. Among them,the majority of mothers said midwives were the most qualified person. On the other hand, some preferred TBAs because TBAs not only did cooking, washing and running errands for the mother and newborn baby

* Upper group consists of those who earn from 1,000 thousands to more than 2,000 thousands Kyat a year; middle group consists of those who earn from 200 thousands to 1,000 thousands Kyat a year; and lower group consists of those who earn from 100 thousands to 200 thousands Kyat a year.

Table 1. Types of birth attendants by place of delivery and social group of family (n = 750)

Social group and place of delivery	Birth attendant (%)*							Total (%)**
	Hospital staff	GP/OG	Midwife	AMW	TBA	Relative	Self-delivery	
Upper group	n = 6		n = 21	n = 5	n = 3			
Hospital	6 (100.0)	0	0	0	0	0	0	35(4.7)
Home	0	0	21 (100.0)	5 (100.0)	3 (100.0)	0	0	
Middle group	n = 22	n = 5	n = 148	n = 17	n = 41	n = 3	n = 4	
Hospital	22 (100.0)	0	0	0	0	0	0	240 (32.0)
GP clinic	0	4 (80.0)	0	0	0	0	0	
Home	0	1 (20.0)	147 (99.3)	17 (100.0)	39 (95.1)	3 (100.0)	4 (100.0)	
Other place	0	0	1 (0.7)	0	2 (4.9)	0	0	
Lower group	n = 29	n = 1	n = 277	n = 46	n = 105	n = 13	n = 4	
Hospital	29 (100.0)	0	0	0	0	0	0	
GP clinic	0	1 (100.0)	0	0	0	0	0	475(63.3)
Home	0	0	275 (99.3)	46 (100.0)	103 (98.1)	13 (100.0)	4 (100.0)	
Other place	0	0	2 (0.7)	0	2 (1.9)	0	0	
Total (%)***	57(7.6)	6 (0.8)	446 (59.5)	68 (9.1)	149 (19.9)	16 (2.1)	8 (1.1)	750

Note: Home delivery = 681 (90.8%), Hospital delivery = 57 (7.6%), GP clinic delivery = 5 (0.7%), other place = 7 (0.9%)

* Percent of each cell total

** Column percent

*** Row percent

Table 2. Basic expense for delivery by types of birth attendant and place of delivery (n = 750)

Birth attendant and basic expense (in Kyats)*	Place of delivery (%)**				Total (%)***
	Hospital	GP clinic	Home	Other	
Hospital staff	(n = 57)				
150 – 5000	2 (3.5)	0	0	0	
5001 – 10000	4 (7.0)	0	0	0	
10001 – 50000	13 (22.8)	0	0	0	57
50001 – 100000	22 (38.6)	0	0	0	(7.6)
100001 – 150000	13 (22.8)	0	0	0	
150001 – 221000	3 (5.3)	0	0	0	
GP/OG		(n = 5)	(n = 1)		
10001 – 50000	0	4 (80.0)	1 (100.0)	0	6 (0.8)
100001 – 150000	0	1 (20.0)	0	0	
Midwife			(n = 443)	(n = 3)	
150 – 5000	0	0	224 (50.6)	1 (33.3)	446 (59.5)
5001 – 10000	0	0	139 (31.3)	2 (66.7)	
10001 – 50000	0	0	75 (16.9)	0	
50001 – 100000	0	0	5 (1.1)	0	
AMW			(n = 68)		
150 – 5000	0	0	29 (42.6)	0	
5001 – 10000	0	0	25 (36.8)	0	68 (9.1)
10001 – 50000	0	0	14 (20.6)	0	
TBA			(n = 145)	(n = 4)	
150 – 5000	0	0	79 (54.5)	3 (75.0)	
5001 – 10000	0	0	41 (28.3)	1 (25.0)	149 (19.9)
10001 – 50000	0	0	24 (16.5)	0	
50001 – 100000	0	0	1 (0.7)	0	
Relatives			(n = 16)		
150 – 5000	0	0	10 (62.5)	0	16 (2.1)
5001 – 10000	0	0	3 (18.7)	0	
10001 – 50000	0	0	3 (18.7)	0	
Self-delivery			(n = 8)		
150 – 5000	0	0	7 (87.5)	0	8 (1.1)
10001 – 50000	0	0	1 (12.5)	0	
Total (%)****	57 (7.6)	5 (0.7)	681 (90.8)	7 (0.9)	750

* Including consultation fee, medicine, transportation, food, fire wood, clothing, etc.

** Percent of each cell total

*** Column percent

**** Row percent

for weeks, but also accepted the fee in instalments. However, the mothers had to spend same total amount (ranged from 150 to 100,000 K) to give birth with mid-wives or TBAs. Those who delivered by TBAs said they used to consult midwives for further medication after birth, which resulted in similar expense as with mid-wives. If they were delivered at private clinic or admitted to hospital, in addition to the basic expenses, they had to spend for operation theatre including health staff and other expenses for the companions. The expenditures then went up to 150,000 K for GP delivery and 221,000 K for hospital delivery (Table 2).

Additional cost

Out of 750 mothers, 537 mothers (71.6%) said they were affected due to absence from work for weeks, months or years during delivery period particularly lacking in income. Of which, the majority (70%) were from the families of lower social group and about 82% of them were working mothers. Thus, during those periods, while some had to employ temporary workers to look after their family domestic work, a few had to use their savings or borrow money. In this case, an additional cost of absence from work was considered. If we included this cost, the maximum expense then increased to 794,000 Kyats. About 74% of them used up to 50,000 Kyats and 25 mothers spent more than 150,000 Kyats.

Influencing factors

Not every mother could afford such high expense of birth delivery and some suffered financial hardship. In addition, some had family problems for delivering at private clinic or hospital. Hence, the majority of mothers preferred home deliveries by their residential birth attendants, and their common reasons were financial problem (un-affordable to deliver at hospital, less charges for delivery), social circumstances (felt secure because of surroundings with family members and relatives, unable to find a person to accompany to hospital), experiences (used to give birth easily) and a local custom of giving births at home.

The findings indicate that midwife was a key person for birth delivery and home delivery was the highest (90.7%) in rural areas of Kyaunkpadaung Township. About 60% of mothers delivered by midwives followed by TBAs and AMWs. The mothers perceived midwife as the qualified person for pregnancy care. However, some mothers gave births mostly with TBAs because of low charges and they were satisfied with their care after birth. Basically, the majority had spent a maximum of 50,000 Kyats with a range from 150 to 221,000 Kyats for delivery. If they were absent from work during delivery period, it increased to 794,000 Kyats. Large amount of basic expenses for delivery was contributed by consultation fee of GP/OG and high cost of hospitalization. The majority of mothers preferred home deliveries by their residential birth attendants, and their common reasons were financial problem, social circum-stances, experiences and a local custom of giving births at home.

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Stigmatization among disabled persons affected by leprosy

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In order to develop strategies for improving social image and restoring self-confidence of persons affected by leprosy (PAL) in the community, this study was conducted to identify social stigma among disabled persons and to differentiate stigmatization between persons disabled due to leprosy and not due to leprosy. The study is cross-sectional comparative design. Aunglan Township, one of the nine areas of JICA disability survey, was purposely selected. Out of registered 150 PALs with GI and II disability, 97 were interviewed during 2004 and 2005. Data collection method was face-to-face interviews using structured questionnaires. In comparison, 97 disabled persons affected by others than leprosy (Non-PAL) were also interviewed. Gender and education levels were not different between PALs and non-PALs. Proportion of married and divorced was higher among PALs than those of non-PALs (61% vs.40% and 7% vs. 3% respectively). Proportions of persons who had a job were not different. Among jobless, PALs gave reasons more with "depending on offspring" and "getting older" while non-PALs gave reasons more with "physically disabled". Male PALs were getting more leadership role in the family comparing to non-PALs (87% vs. 49%). During the festive occasions, slightly higher proportion of "non-invited" among non-PALs than PALs was found. The responses shows obviously high level of self-stigmatization among both PALs and non-PALs but two groups were not significantly different. Similar patterns of stigmatization were found in the persons during social occasions and village affairs. About 10% higher in PALs than non-PALs on perceived being-discrimination shows that PALs might have self-stigmatization due to the disabilities affected by leprosy. Findings highlight stigma of PALs might not be caused by physical disabilities but by disfigurement or name of disease "*leprosy*". And also, prevention of disfigurement and psyco-socio rehabilitation is crucial for improvement of quality of life of PALs.

INTRODUCTION

Leprosy is noted as an endemic disease in Myanmar for many centuries [1]. It has been depriving both patients and their families of leading a normal productive life. Leprosy is an ordinary disease with extraordinary social and economic implications. More numbers of studies do not adequately express the social and economic loss [2]. Leprosy and its consequences are a complex human problem leading to discriminations, stigma and prejudices [3, 4].

Though the impact of social inequality on health conditions is widely known, its impact on the chronic and stigmatized disease, leprosy, has received little attention. Deformity sometimes leads to physical disabilities and to handicaps consequently causing problems to the patient and his family.

Stigma is a process of behavioural responses of community who are relating to an affected person. The process of stigma-tization can be divided into two stages. The first stage describes how certain cognitive dimensions of leprosy lead to a variety of affective responses towards the disease. The second stage involves how these affective responses contribute to social devaluation of the leprosy patient and consequently, the adoption of negative behaviours towards them [5]. Stigma acts as a factor of socio-economic consequences of disabled persons affected by leprosy. Many studies pointed that leprosy is causing various socio-economic problems and many other problems due to the physical disabilities resulting from the disease. However, there is no clear determination that whether socio-economic consequences of persons affected by leprosy (PALs) are due to leprosy disease or physical disabilities. Low social image among PALs have to be approved whether it is due to disease itself or deformity. This study was conducted mainly to identify social stigma among disabled persons and to differentiate stigmatization between persons disabled due to leprosy and not due to leprosy (PAL and non-PAL).

These findings will be used as an input for Leprosy Control Program in order to develop strategies for improving social image and restoring self confidence of affected persons in the community. Integration between Prevention of Disabilities (POD) and Community Based-Rehabilitation (CBR) should be promoted to enhance the quality of lives of PALs and non-PALs in community.

MATERIALS AND METHODS

The study is a cross-sectional comparative design. After detailed discussion with the concerned health persons from Leprosy Control Programme, the study group should be in the area where the rehabilitation programme by JICA has being initiated. Therefore Aunglan Township, one of the nine areas of JICA disability survey was purposely selected to integrate two projects (the present project and JICA project) so that the needs of the leprosy patients can be provided effectively in intervention phase. There were 150 registered GI and GII PALs at the township of study. Recruitment of the GI and II cases and identification of their social surroundings was done by the township leprosy control programme. Data collection was done during 2004 and 2005. Data collection method was face-to-face interviews using semi-structured questionnaires. Trained interviewers were used for interviews. Out of registered 150 PALs with GI and II disability, only 97 were interviewed due to difficulties in finding cases that were living in the forest, moving out and hospitalized for reconstruction. In comparison, 98 disabled persons affected by others than leprosy (non-PAL) were also interviewed.

RESULTS

Background characteristics of study subjects

Among the total 195 subjects, majority (63%) were males and remaining one-third were females. Male-female proportions were not different between PALs and non-PALs (male was 63% & female was 37% in both groups) (P=0.956).

Regarding educational status, majority (54%) were at middle school level. Nearly one-third (31%) were lower than middle school level. Distribution of persons with different educational levels was not significantly different between PALs and non-PALs (P=0.297).

There was a significantly different pattern of distribution of marital status between two groups (P=0.001). The proportion of "singles" was higher among non-PALs than PALs (39% vs.15%) while that of "married" was higher among PALs (61% vs. 40%). Proportion of "divorced" was two-folds higher among PALs than non-PALs (7% vs. 3%). Age distributions were not significantly different between two groups.

Type of disabilities

Differences of type of disabilities were observed between two groups. Skin disfigurement was obviously high between PALs (78.4%). Other organs affected (eye, hand and foot) were higher among non-PALs in comparing to PALs (29.6% vs 3.1% for eye, 13.3% vs 2.1% for hand, 37.8% vs 1.0% for foot respectively) (*Pearson* $Chi^2(6) = 148.8671, P < 0.001$).

Occupational status

There was no different proportion of having job at the time of interview. The majority gave reason "due to physical disability" (55%). However, reasons for not having a job were found different among groups. Higher percentages of PALs gave reasons of "depending on offspring" and "getting older" are found (21% vs. 2% and 15% vs.6% respectively) (Table 1).

Table 1. Reasons for not having a job among PALs and non-PALs

Job status	Non-PALs		PALs		Total	
Having a job*	Freq	%	Freq	%	Freq	%
Yes	43	44.3	44	45.4	87	45.1
No	53	55.7	53	55.6	106	55.9
Total	96	100	97	100	193	100
Reasons for not having a job**						
Due to disability	37	68.5	21	40.4	58	54.7
Weakness/Fatigue	6	11.1	7	13.5	13	12.3
Offspring are earning	1	1.9	11	21.2	12	11.3
Getting older	3	5.6	8	15.4	11	10.4
Still schooling	4	7.4	2	3.9	6	5.7
Never worked	3	5.6	3	5.8	6	5.7
Total	54	100	52	100	106	100

* Pearson chi^2 (1) = 0.0063, Pr = 0.937
** Pearson chi^2 (5) = 15.7313, P = 0.008

Income

Although they were not statistically significant, mean and median income of PALs were higher than non-PALs (14450 kyats vs 12775 kyats and 10000 vs 9000 kyats respectively). Similarly, total family income in kyats per month was also higher in the families of PALs than that of non-PALs (26367 vs 22007 kyats and 23146 vs 18124 kyats respectively).

Family leader role of persons

Getting "family leader role" of female persons in this study was higher among PALs than non-PALs (25% vs. 14%) but it was not statistically significant. However, among male persons PALs were getting more leader role in the family in comparing to non-PALs (87% vs. 49%) (P<0.001).

Social events in festive occasions

During the festive occasions, disabled persons might have some socially stigmatizing experiences. Three sequential questions were asked to the persons. Do they have invitations to such occasions like other people? Slightly higher proportion of "non-invitees" among non-PALs than PALs was not significant (P=0.429).

The second question was "Did they go to this occasion?" to the persons who received invitation. The answer "no" was noted as "self-stigmatized". Like the first question, PALs and non-PALs were not significantly different (P=0.246). However, the third question "Did they experience social problem during their presence in the occasion?" figured out non-PALs have more problem than PALs (P=0.017). Among three questions, the responses show obviously high level of self-stigmatization among both PALs and non-PALs. Among those who did not go to the occasion although invited, the reason for not going were explored. Proportion of not willing to go to social occasions was 74.5% among PALs and 8.2% among non-PALs. Some 9.3% of PALs and 7.1% of non-PALs said they were not feeling well at the time of occasion. Reason of "low esteem" was given by 9.3% of PALs and 1.2% of non-PALs (Fig. 1).

Social events in social occasions

To explore stigmatization on social occasions, similar questions were asked. The responses showed that non-PALs were less invited than PALs (P=0.017). For

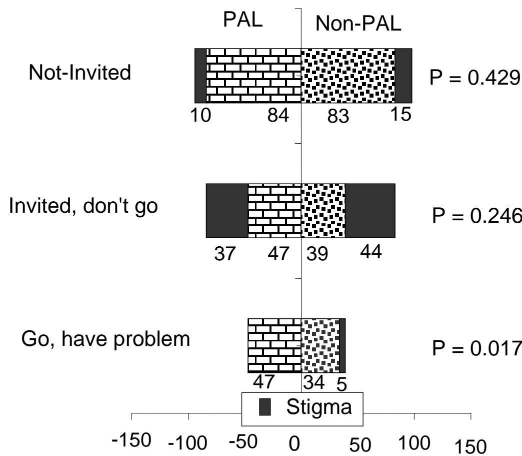

Fig.1. Comparison of PALs and non-PALs having stigma events on festive occasions

the next two questions the responses were not different among two groups. Self-stigmatization was also pronounced like in festive occasions. About 30% of PALs felt "reluctant to go to the social occasion" while 44% of non-PALs gave such reason. "Low esteem" was given as a reason for not going by 14.8% of PALs but no non-PALs said like this (Fig. 2).

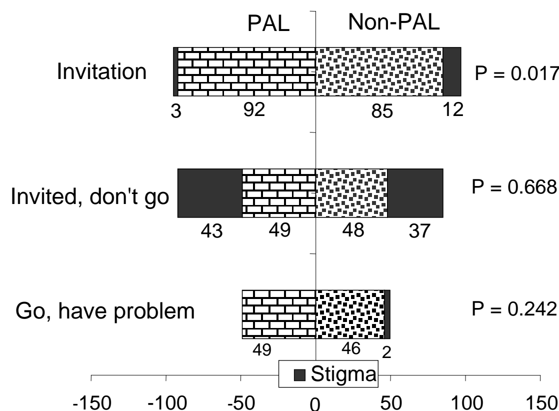

Fig. 2. Comparison of PALs and non-PALs having stigma events on social occasions

Social events on village activities

To explore stigmatization on village occasions, similar questions were also asked. The responses show that PALs were not less stigmatized than non-PALs on all three questions. Self-stigmatization was also pronounced like in festive occasions. Although majority, both PALs and non-PALs said they were not free to participate, 15% of PALs and 22% of non-PALs said they did not want to go there. Another 15% of PALs and 22% of non-PALs also responded that they did not go to avoid people. Feeling low esteem was also given as a reason for not going by 8% of PAL and 17% of non-PALs (Fig.3).

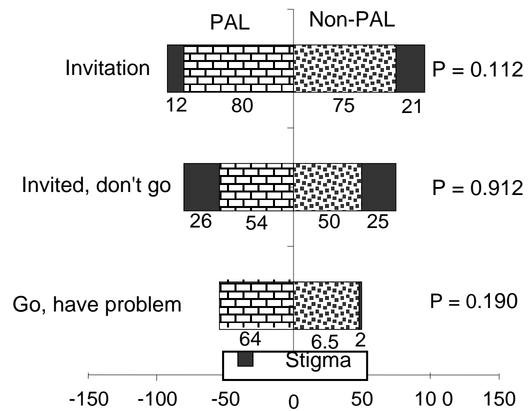

Fig. 3. Comparison of PALs and non-PALs having stigma events on village activities

Perceptions of PALs and non-PALs on response of villagers

PALs and non-PALs were also compared on their self-stigmatization with perceived discrimination of other people on them. Forty-eight percent of non- PAL and 58% of PALs noticed that other peoples' behaviour on them which looked discriminating. About 10% higher in PALs than non-PALs on perceived being-discrimination shows that PALs might have self-stigmatization due to the disabilities affected by leprosy (Chi square 17.95, p=0.001). However, second question "What is the response of villagers towards them?" was asked to highlight discrimination. A non-response rate on this question was high (about 40%). No difference was found between PALs and non-PALs (P=0.287) (Table 2).

Table 2. Responses of villagers on disabled persons

Response of villagers	Non-PAL		PAL		Total	
	Freq	%	Freq	%	Freq	%
As usual	30	30.61	42	43.30	72	36.92
Friendly	8	8.16	5	5.15	13	6.67
Discriminate	9	9.18	8	9.28	18	9.23
Not answer	51	52.04	41	42.27	92	47.18
Total	98	100.00	97	100.00	195	100.00

Pearson Chi^2 (2) = 3.7742, P = 0.287

DISCUSSIONS

Proportion of ever-married among PALs was higher than non-PALs since leprosy might not affect them at their early age of marriage. However, higher divorce rate among PALs may have some explanation to be uncovered. It highlights leprosy might be a factor for marital problems among disabled persons.

Family income or personal income of PALs was not different from that of non-PALs. Major disability for PALs might not be due to physical disablement but due to disfigurement which may affect them socially disabled. Having social disablement was noted among both PALs and non-PALs. Rejections from environment and reluctancy to environment of the affected persons were carefully analysed. Intra-family role of PALs was not lower than that of non-PALs. It means that PALs could lead their family by generating income. They get acceptance of their family.

A high level of self-stigmatization among leprosy patients was also observed in India and equally a high level of social stigma was found in their communities, which led to reduced interaction between the leprosy patients and their communities [6]. That study did not mention discrepancy of stigma between PALs and non-PALs. In a study of community perception in high and low endemic region in Myanmar, about 44% of community had positive attitudes towards PALs showing leprosy was still a social stigma [7].That study did not mention that whether those who had negative attitudes towards leprosy were due to leprosy disease or disability of the patients. In a study on social aspect of leprosy in Hmawbi Township, Myanmar, about 8% of patients felt that they were outcasted by society [8]. The study did not describe the differential of outcasting among the patients with and without disability. In our study, getting invitation, participation and discrimination at extra-familial environment were not significantly different between PALs and non-PALs. Higher percentage of self-stigmatization (refusing invitation to participate) was noted among PALs at social occasions and community activities but this was not much. It shows that stigma among PALs might be not due to "leprosy" but due to disfigurement (consequence of disease). This statement was supported by qualitative findings from another part of this study [9]. PALs do not want to be known as leprosy cases. Community acceptance is an issue for leprosy cases and mentioned that leprosy patients especially disabled cases used to stay away from people and they have low self esteem. The patients fear that neighbors might know of taking treatment for leprosy.

Prevention of disfigurement of PALs will be more important in future because decreasing incidence and improved case management programme including POD and POWD would minimize physical disablement among PALs remaining main cause of social disablement and stigma of PALs as disfigurement. There is lack of complete understanding about global needs for rehabilitation in current situation.

In Myanmar, Community Based Rehabilitation (CBR) has been initiated mainly based on physical and health grounds. Concentration on medical care of people affected by leprosy (Multidrug therapy MDT, surgery, etc) will be vastly beneficial. However, psycho-socio-economic rehabilitation is a holistic manner which will reduce self-stigmatization among disabled persons thus resulting in improvement of quality of life.

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Use of in-house test system for investigating prevalence of *Plasmodium falciparum* antisporozoite antibodies in a malaria endemic area

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Malaria ranks as first priority disease in Myanmar. For effective control of malaria, the situation of malaria transmission in the area must be known as much as possible. Of several indicators, antisporozoite antibody is considered as a useful indicator for assessing the transmission and could be used in planning the progress of vector control programmes. We developed an in-house Indirect Enzyme Immunosorbent Assay (EIA) system using synthetic peptide $NANP_3$ as the solid phase and peroxidase labeled anti-human IgG (Rabbit) as the conjugate. Checkerboard titration was carried out and the dilutions determined. The antisporozoite antibody levels of 384 subjects from Tarchileik (mean age 34.71 ± 19.2yrs) were determined. The mean antibody levels ranged from 0.08μg to 21.9μg. No significant difference in the anti-sporozoite antibody positive rate was found between males and females (29.2% *vs* 37.6%). A positive correlation ($r = 0.32$) with age ($p<0.001$) was found and highest antisporozoite antibody positive rate was found in 20-40 years age group followed by 40-60 years age group. No association was found with history of malaria. The developed EIA could be used to assess the degree of malaria transmission in a locality.

INTRODUCTION

The detection of antibodies to sporozoites in the sera of individuals living in malaria endemic areas is of epidemiological relevance, since antibody levels should correlate with the intensity of transmission of the disease. In persons living in areas with endemic malaria, prevalence and levels of sporozoite antibodies have been shown to correlate with the entomologic inoculation rate at the same time for the same area. The immunodominant epitope of the *Plasmodium falciparum* CS protein consists of highly conserved tandem repeats of amino acid (Asn-Ala-Asn-Pro=NANP) [1].

An EIA using a chemically synthesized $NANP_3$ peptide has become available [2]. It has been possible to screen large scale samples in shorter interval than needed for salivary gland dissection, and also with greater sensitivity and specificity results. Circumsporozoite (CS) antibodies indicating the occurrence of malaria infection but not necessarily development of disease, have been shown to be reliable indicators of transmission in area with disease [3,4,5]. The results obtained from the in-house EIA test will be applicable in assessment and monitoring of malaria transmission in a locality as well as in planning the control measures of malaria [5,6,7].

MATERIALS AND METHODS

The laboratory-based experimental study was conducted in the Nuclear Medicine Research Division as mentioned below. This producer as reported by M.Knappik was modified for local situation [8]. One-hundred microlitre of $NANP_3$ (1µg/ml~10µg/ml) in PBS was added to polystyrene microtitre plate (NUNC, Germany), incubated at 37˚C for (1~5 hr) and following 4˚C overnight and washing with PBS+0.1% Tween 20. Remaining protein binding sites were blocked by different concentrations of 0.5 ~10% BSA fraction V in 0.15 M PBS pH 7.2 with addition of Tween 20 for varying incubation time from 10 minutes to 2 hours. Following extensive washing with PBS-Tween 20, different sporozoite antibody standard concentrations (0.625 to 5 µg/ml) in PBS or Normal Human Serum plus PBS were incubated for 1 to 2 hours at 37˚C and then washed. Normal Human Serum was used as a negative control. Addition of 100 µl of peroxidase conjugate rabbit anti-human IgG (1:1000~1:10000) in PBS for 1 hour was followed by washing and incubation with substrate solution (40 mg of orthopheny-lenediamide dissolved in phosphate-citrate buffer, pH 5.0) for 30 minutes. The reaction was stopped by adding 2.0 M H_2SO_4. The results were read at 492 nm micro plate reader (Thermal Labsystem Multiskan EX).

Preparation of standard curve

Standard curve was developed by testing circumsporozoite antibody concentrations ranging from 0.625 to 5.0µg/ml.

Study population

All 384 subjects in this investigation were recruited in February 2006 from Tarchileik Township. Serum specimens were stored at -40˚C. The $NANP_3$ EIA with pre coated EIA plates (NUNC, Germany) was used to perform the method developed in our lab. All specimens were screened at 250-fold dilution in duplicate wells. Two negative reference control sera and positive reference control sera confirmed by Immunoradio-metric assay method were included on each plate as quality controls. Results were obtained by use of a micro plate reader (Thermal Labsystem Multiskan EX) with a wavelength capability of 492 nm. A calibration curve was derived by plotting the absorbance values of the calculation sera on linear paper versus defined unit values (µg/ml). The antibody levels of the specimen were determined by interpolation of the absorbance mean of each serum on the calibration curve. Statistical analysis was done using Minitab 14.1 software packages.

RESULTS

The optimal concentration of circumsporo-zoite antigen $NANP_3$ 100 µl used for coating the plate is observed to be 2.5µg/ml.Optimal blocking with 5%BSA-PBS was achieved after 1 hour incubation at 37˚C.

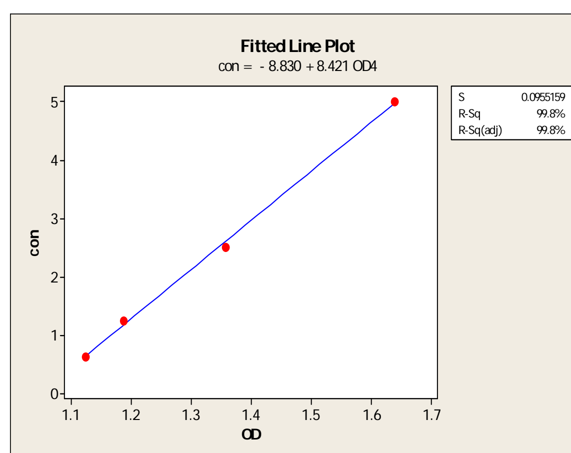

Fig.1. Regression Analysis: con versus OD

The regression equation is
con = - 8.830 + 8.421 OD4
S = 0.0955159 R-Sq = 99.8% R-Sq(adj) = 99.8%
Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	11.2122	11.2122	1228.97	0.001
Error	2	0.0182	0.0091		
Total	3	11.2305			

Fitted Line: con versus OD

The test showed the sensitivity level of detecting circumsporozoite antibody 0.625 µg/ml. The optimal time for incu-bation with 1:1000 peroxidase labeled

antibody conjugate was achieved after 1 hour. The standard curve was obtained as shown in Fig 1.

Table 1. Detection of circumsporozoite (CS) antibody of *P. falciparum* among the subjects in Tarchileik.

Sex	No. of subjects	No. of CS antibody positive (%)	No. of CS antibody negative (%)
Male	113	33 (29.20)	80 (70.80)
Female	271	102 (37.64)	169 (62.36)
Total no. of samples tested	384	135 (35.2)	249 (64.8)

Of the 384 investigated subjects, 113(33.3%) were males and 271(66.7%) were females. The average age was 34.71±19.2 years. By use of calibration sera provided with the test, the cut-off value for measurement of CS antibodies was defined as 7.38 µg/ml. Serum specimens from 135 (35.2%) of the 384 subjects were positive and 249 (64.8%) were negative. No significant difference in the antisporozoite antibody positive rate was found between males and females (Table 1). A positive correlation (r=0.32) with age (p<0.001) was observed and the antisporozoite antibody positive rate was highest in 20-40 years age group followed by 40-60 years age group (Fig. 2).

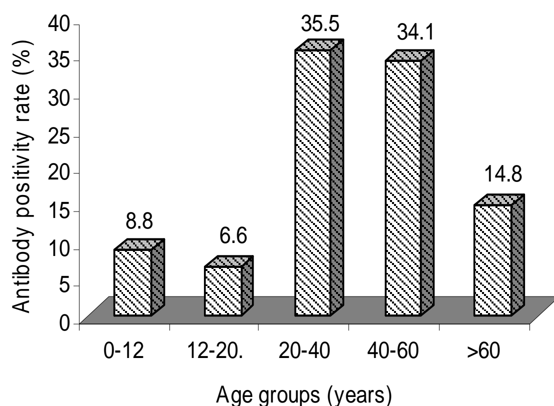

Fig. 2. Age specific circumsporozoite seropositivity rates in Tachileik

DISCUSSION

After performing trial and error assays of EIA system, by using different incubation times, concentrations of $NANP_3$, and conjugate dilution, a reliable and applicable in-house test system of EIA for detecting *Plasmodium falciparum* antisporozoite antibodies was able to develop. Circumsporozoite antigen $NANP_3$ in the concentration of 2.5 µg/ml was needed to coat the polystyrene microtitre plate. Conjugate incubation time for 1 hour with 1:1000 peroxidase labeled antibody conjugate (rabbit anti-human IgG) was necessary to produce a sensitivity level of detecting 0.625 µg/ml circumsporozoite antibody in the human serum. According to the results obtained in the study, it was evident that there is a considerable transmission of malaria in Tarchileik. 35.2% (135) of the study population was recently exposed to malaria infection, in which 29.2% (33) males and 37.64% (102) females were observed to have recent or previous infection. Although statistically not significant, the females were found to have higher infection rates than those of males. The mean antibody levels ranged from 0.08 µg to 21.9 µg. There was no significant difference in antisporozoite antibody positivity rate between males and females. But a positive correlation (r=0.32) with age was found and antisporozoite antibody positive rate was observed to be highest (35.56%) in 20-40 years age group and second highest (34.07%) in 40-60 years age group. It indicated that 20-40 years age groups got highest exposure to malaria infection. 40-60 years age group had second highest exposure to malaria infection. Being 20 - 40 years age group is the most active and most mobile population in that area, the more chance to expose to the malaria infection so that the higher antibody positivity was observed. The second most active population of 40–60 years age group was also evidently found to have the second highest antibody positivity rate. Therefore it can be concluded that the age specific anti-sporozoite antibody positivity rate can be a good indicator for assessing the malaria transmission potential among the population. On analysis, there was no association

observed between a antisporozoite antibody level and history of clinical malaria in this study. In conclusion, the EIA system developed in-house in Nuclear Medicine Research Division could be used as an adjunct in assessment of malaria transmission in a locality where other malariometric diagnoses are not possible.

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