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Diagnosis of *Plasmodium falciparum* infection from two different endemic areas by two immunological methods using glycopospholipid antigen

*M. M. Mya, ***R.K. Saxena, ****A. Roy, *****K. B. Roy, *W. Tun Lin, *Sein Min,
*Sein Thaung, *Sein Maung Than & **Kyaw Zaw

*Medical Entomology Research Division, DMR(LM)

** Vector Borne Diseases Control

*** Centre for Biomedical engineering IIT New Delhi-110016, India

**** Malaria Research Centre, 22 Shamnath Marg, Delhi 110054 India

***** Centre for Biotechnology, Jawaharlal Nehru University- New Delhi 110067, India

Previously described isolated Glycophospholipid (GPL) antigen's diagnostic sensitivity and specificity properties were tested on malarial and non malarial blood samples from two different malaria endemic areas in Myanmar and India. The control area is a non-endemic area, Delhi, India. Standard Enzyme-Link Immunosorbent Assay (ELISA) and modified Laser Immunoassay (LIA) methods were used. The crude parasite rates were found to be 28.75% in Assam, India and 32.5% in Taikkyi, Myanmar respectively. *P. falciparum* was predominantly high in both forested-hilly areas. Immunoreactivity and specificity of GPL antigen were compared with those of another previously reported RESA derived synthetic peptide antigen (R1). The diagnostic sensitivity properties of GPL antigen were found cent percent sensitive for *P. falciparum* cases in both areas by LIA method but ELISA with R1 antigen showed a slightly lower level of sensitivity than GPL antigen. The specificity studies of GPL and R1 antigens tested on malarial and non malarial diseases sera were found to be very specific for *P. falciparum* as they did not react with other non malarial diseases sera. LIA method with the application of GPL coated latex beads can be an excellent diagnostic tool for *Plasmodium falciparum* infection.

INTRODUCTION

Traditional diagnosis of malaria by microscopic examination of blood smears is an excellent and efficient method, but is highly dependent on human skill and diligence. This human factor has probably been the cause of underreporting of malaria worldwide. As an alternative, sensitive immunoassays like ELISA, radio immunoassay (RIA), Indirect immuno-fluorescent antibody test (IFA) and Opti-Mal tests have been developed and tried for mass application [1,2,3]. These diagnostic methods are quite specific and sensitive but their procedures are very lengthy and expensive. Agglutination assay is the

simplest and cheapest immunoassay but has not been employed because of its low sensitivity as the antigen antibody complexes are often microscopic and not visible to the naked eye. Laser immunoassay (LIA) based upon laser light scattering can detect microscopic agglutinates and can raise the sensitivity of agglutination assay enormously [4,5]. Recently, development of data analysis by CONTIN method in dynamic light scattering [6] led us to develop LIA for malaria [7], which is as sensitive as ELISA but much simpler in principle and practice. Here we report specific diagnosis of *Plasmodium falciparum* infection by LIA using a new glycopospholipid antigen

isolated from *Plasmodium falciparum* culture supernatant. We demonstrate here that the GPL antigen is very specific and can identify positive infection in clinically diagnosed patients both from India and Myanmar.

MATERIALS AND METHODS

Antigens

The synthetic peptide antigen R1 [8] was gifted from Cambridge Bioresearch Co. UK., and the GPL antigen was isolated from *Plasmodium falciparum* culture supernatant. Isolation of GPL antigen is described briefly as follows:

A single *Plasmodium falciparum* isolate, PSJM, from Sahajahanpur, U.P. India was adapted to continuous culture as described by Trager and Jensen [9]. One litre of healthy culture having parasitemia above 5 percent was selected. Spent culture supernatant was collected after removal of parasites. Supernatant was concentrated, then dialysed against water and lyophilized as a powder. The powder is extracted with chloroform and the chloroform extract yielded a residue, which was characterized as a glycopospholipid having xylose, mannose, galactose and glucose in its sugar moieties. Details of isolation and characterization will be reported elsewhere [10]. To test the parasite origin and immunoreactivity, it was tested against human sera of slide positive cases of *Plasmodium falciparum*, *Plasmodium vivax* as well as sera of healthy individuals with no history of malaria, as negative control.

Background of study area

Control group

Thirty blood samples from infants (aged between 2 months to 2 years with no history of malaria) were collected from Delhi hospitals by finger prick method on 3mm thick whatman filter paper. Thick and thin

blood smears on glass slides were also prepared for microscopic examination to confirm the absence of malarial parasites and served as negative controls.

Test group

Assam: It is a northeastern state of India. The area is hilly, covered with dense forest, and is highly endemic for malaria, predominantly *Plasmodium falciparum* malaria. Population is mostly tribal dependent on forest. Extensive use of bed nets apparently has reduced man-mosquito contacts.

Myanmar: Yeasikan village, Taikkyi Township is in a hilly area situated by the site of the Gyophyu Dam. Malaria transmission is perennial because of mosquito breeding in the water body of Dam. Villagers are mostly dependent on forest products and negligent about malarial drugs and other protection measures.

Collection of test blood samples

Thick and thin blood smears on glass slides were prepared from blood samples of fifty each of Assam and Myanmar human subjects of fever cases and were examined microscopically after Giemsa staining for positive, negative and species identification of parasites. After having written informed consent from all the subjects, finger prick blood was collected from microscopically *P. falciparum* positive cases on filter paper discs, which were stored at -20° C until used for serological examination.

Collection of non malarial blood samples

Ten samples each of six common non-malarial sera (HIV, TB, Asthma, Filariasis, Pregnancy, Normal) were obtained from Safdarjung Hospital and AIIMS, New Delhi for specificity study. This study was approved at Malaria Research Center under ICMR, Government of India.

ELISA and LIA

Methods of Enzyme link immunosorbent assay (ELISA) and Laser light immuno-

assay (LIA) using R1 antigen have already been described [8,7]. ELISA results were read on ELISA reader at 492nm wavelength from Organon Teknica, Belgium. LIA was done on a homemade laser scattering set up. Agglutinations of antigen coated polystyrene beads (90nm nano particles) with antibody present in subject serum is monitored by laser light scattering. The agglutinates can be detected microscopically.

Statistical analysis of data

Data obtained from the study were statistically analysed by paired student 't' test method. Mean, standard deviation and cut-off values (Control mean +2SD) were calculated for sensitivity measurement.

against control blood samples by ELISA and Laser immunoassay (LIA) methods. Out of thirty finger prick control negative blood samples collected on filter discs, all samples were tested to determine the cut-off value (control mean +2SD) for determination of malaria positive and negative cases. The cut-off values of GPL and R1 antigens are as follows i.e. 106.07 and 108.5, which were obtained from LIA method. Cut-off values for ELISA method were determined and found to be 0.2416 for GPL and 0.2642 for R1 antigens.

Test group

Microscopic examination

One hundred (Fifty each from Assam and Myanmar) finger prick blood samples were

Table 1. Diagnosis of Assam and Myanmar blood samples for malaria by LIA , ELISA and Microscopy

Category	Microscopic diagnosis		Laser Light Immunoassay				ELISA			
	Thick blood film exam:		GPL-Antigen		R-1 Antigen		GPL-Antigen		R-1 Antigen	
	A	B	A	B	A	B	A	B	A	B
<i>P. falciparum</i>	23	26	23 100%	26 100%	21 91.30%	24 92.31%	22 65.65%	25 96.15%	21 91.30%	23 88.46%
<i>P. vivax</i>	6	4	---	--	5 83.33%	3 75%	---	-	3 50%	2 50%
Negative	21	20	21 6	20 4	21 3	20 3	21 7	20 5	21 5	20 5
False negative										
Total positive	29	30	23	26	26	27	22	25	24	25
Total negative	21	20	27	24	24	23	28	25	26	25
Control negative (0-2 yrs)	30		30		30		30		30	
Mean $\pm$ SD			(98.36 $\pm$ 3.8532)		(98.72 $\pm$ 4.89)		(0.1954 $\pm$ 0.0231)		(0.1524 $\pm$ 0.0559)	
Cut-off value (Control Mean+2SD)	----		106.07		108.5		0.2416		0.2642	
P value			P<0.001		P<0.001		P<0.001		P<0.001	

RESULTS AND DISCUSSION

Results of LIA and ELISA methods evaluated by GPL and R1 antigens are shown in Table 1.

Control group

Initially immunoreactivity properties of GPL and R1 antigens were measured

used for detection of positive and negative cases of malaria in this study. Microscopic examination of thick and thin Giemsa stained slides showed *Plasmodium falciparum* positive in 49 samples and only 10 blood films showed presence of *Plasmodium vivax*. Remaining 41 blood samples were microscopically negative for malaria.

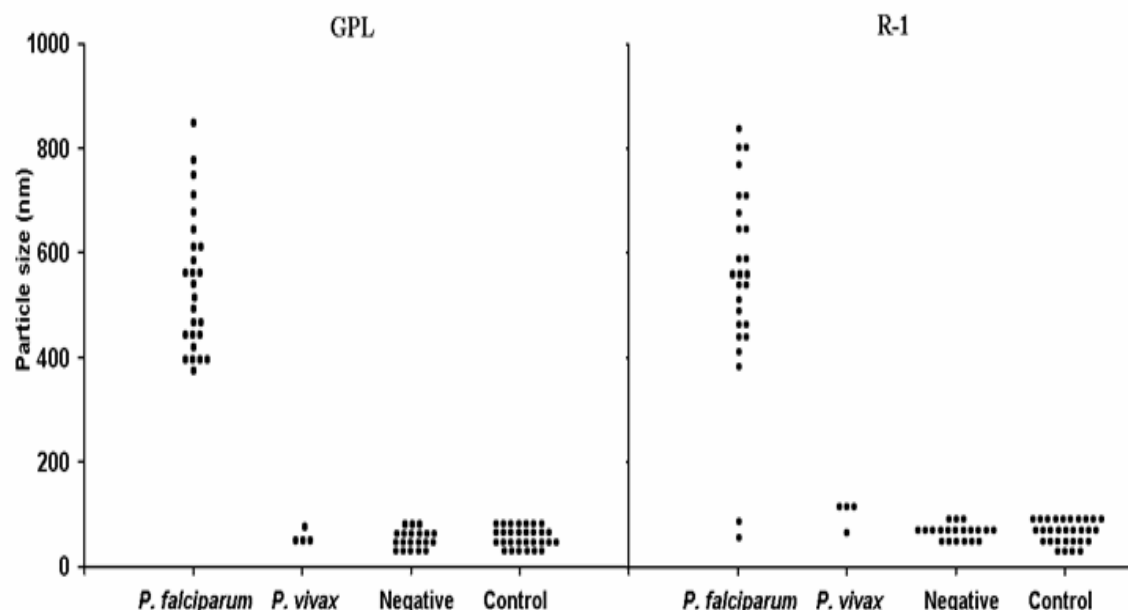


Fig. 1. Estimation of anti-GPL and anti-R1 antibodies by LIA method, samples from Myanmar (Cut-off value-GPL=106.7nm, R1=108.5nm)

LIA method

Detection of malaria in test group by LIA method showed that GPL antigen can detect 49 blood samples of *Plasmodium falciparum*, but R1 antigen could detect only 45 cases of *Plasmodium falciparum* and 8 cases of *Plasmodium vivax* malaria.

ELISA method

Test group blood samples assayed by ELISA method using GPL antigen showed 47 samples *Plasmodium falciparum* positive, however, R1 antigen could detect 44 *Plasmodium falciparum* and 5 *Plasmodium vivax* positive cases.

The results showed that anti GPL titres for *Plasmodium falciparum* cases were about eight to nine folds higher than of *Plasmodium vivax*, negative and control sera ($P<0.001$) but *Plasmodium vivax* titres showed no significance difference between negative and control sera by LIA method. When anti-R1 titres were studied by LIA method, *Plasmodium falciparum* and *Plasmodium vivax* cases were about eight folds ($P<0.001$) and 1.5 folds higher ($P<0.05$) than negative and control sera respectively. The results of ELISA showed

that anti GPL titres for *Plasmodium falciparum* cases were about four to five fold higher than *Plasmodium vivax*, negative and control sera but anti R1 titres for *Plasmodium falciparum* and *Plasmodium vivax* cases were about four to five fold ($P<0.001$) and 1.5 fold higher than that of negative and control sera ($P<0.05$).

Such a distinctive difference in results prompted us to find out a potential immunodiagnostic method with higher sensitivity for *Plasmodium falciparum* more systematically by using two different antigens, R1 and GPL in parallel, tested in two different methods, ELISA and LIA. Such a comparative study between ELISA and LIA using R1 as antigen has already been published [7]. We showed that LIA has actually little edge over ELISA in terms of sensitivity and cost.

In this report a new glycopospholipid antigen isolated from *Plasmodium falciparum* culture supernatant was subjected to a similar comparative study and R1 was also included parallely for comparison. Fig.1 gives the LIA results and Fig. 2 the ELISA results obtained with sera

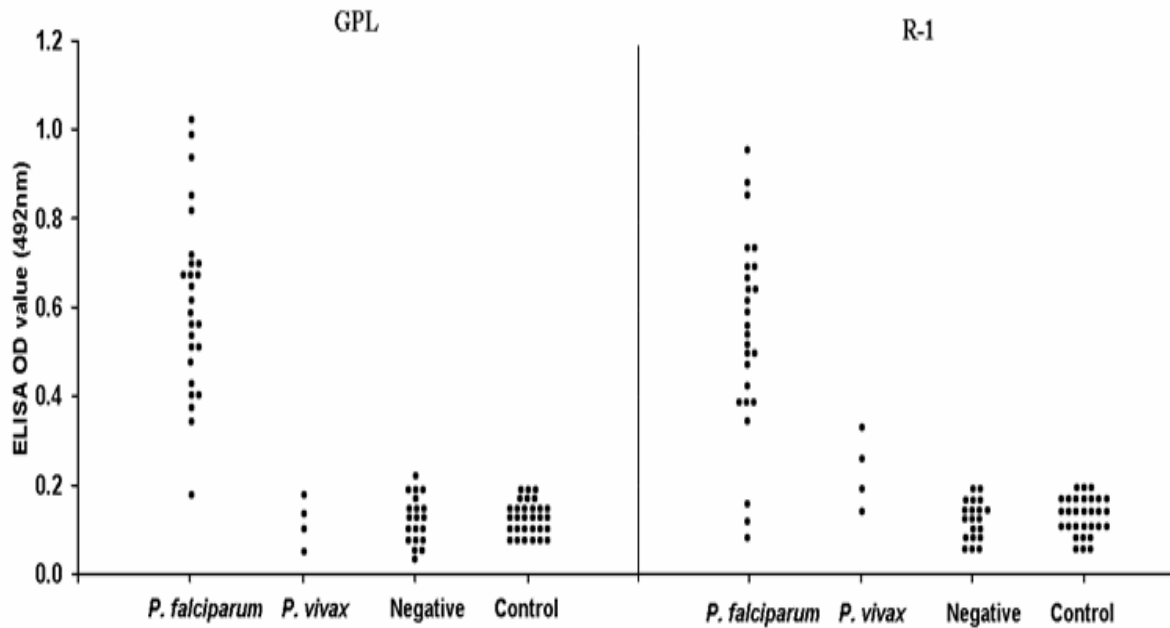


Fig. 2. Antibody titers against GPL and R1 - peptide in the same samples from Myanmar by ELISA (Cut-off value GPL=0.216, R1=0.2642)

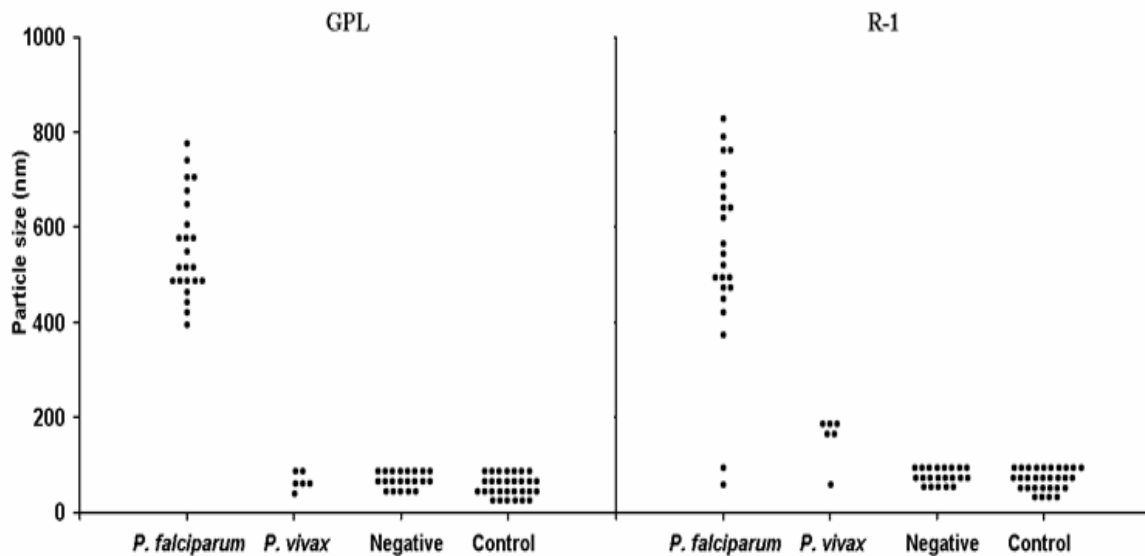


Fig. 3. Estimation of anti-GPL and anti-R1 antibodies by LIA from Assam, India samples (Cut-off value-GPL=106.7nm, R1=108.5nm)

from Myanmar. The ELISA data with R1 antigen are similar as that reported earlier [7]. A large spread in the titre with more overlap between the *Plasmodium vivax* sera and the control sera is evident. The data with GPL antigen are more discriminatory between *Plasmodium falciparum*, *Plasmodium vivax* and negative cases. Figure 1 shows the results of LIA with the same samples, GPL cannot distinguish between the *Plasmodium vivax* and negative controls,

but anti-GPL titres in *Plasmodium falciparum* sera are much higher than the titres in the *Plasmodium vivax* and negative control sera. This indicates that GPL is of *Plasmodium falciparum* origin and is specific. The results with blood samples from Assam, India are given in the Figure 3 and 4. Assam blood samples results showed similar trend as it was observed in Myanmar test group. These results further prove our point of contention that GPL antigen has

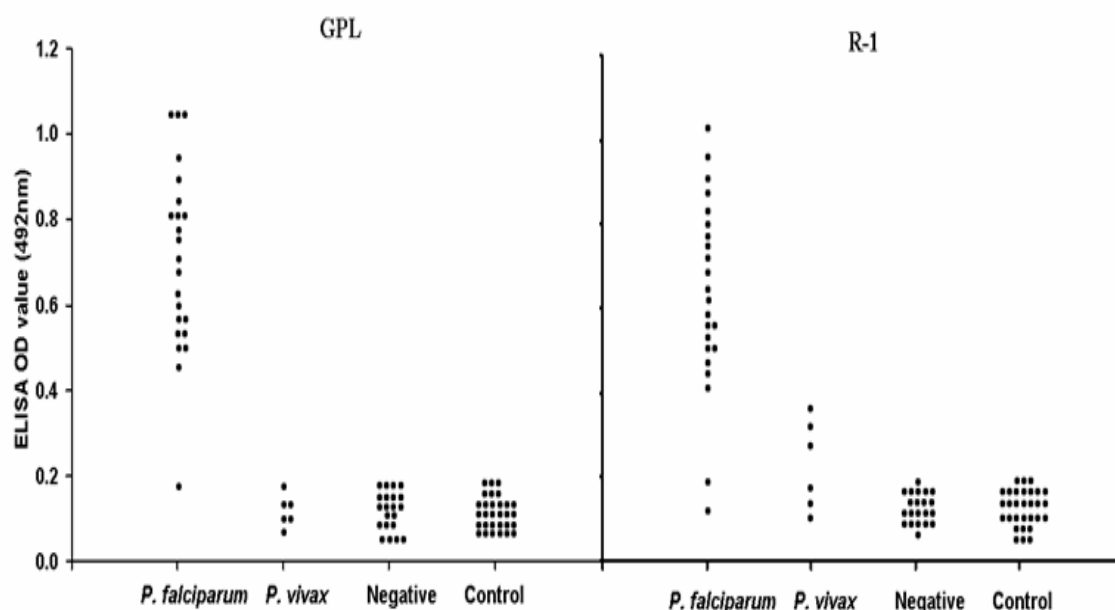


Fig. 4. Antibody titers against GPL and R1 - peptide in the same Assam samples by ELISA (Cut-off value GPL=0.216, R1=0.2642)

better immunodiagnosing reactivity than R1 antigen for *Plasmodium falciparum* malaria as determined by LIA which can be again observed in Figure 3.

We tested the specificity of GPL and R1 antigens also. The results of LIA and ELISA using GPL coated latex beads and non-malarial sera showed no reactivity. The coated beads in absence of sera showed an average diameter of 85-95nm only. No agglutination was shown upon addition of any of the non-malarial sera, which suggests that GPL is non-cross reacting with antigens of other pathogens tested here.

High specificity and discriminatory capacity of GPL antigen for *Plasmodium falciparum* are highly suggestive of its parasite origin. Glycophospholipids synthesis by *Plasmodium falciparum* has been identified [11]. The chemical structure of glycoposphatidylinositol (GPI) of intra-erythrocytic *Plasmodium falciparum* has now been determined including the core glycan [12]. It has been reported that adults who have resistance to clinical malaria contain high level of anti-GPI antibodies whereas, individuals not exposed to the malarial parasite completely lack anti GPI antibodies. It may be noted that Perlman and colleagues reported a blood stage

Plasmodium falciparum antigen using an invasion inhibitory monoclonal antibody (MabA52A6) obtained from clinically immune donor [13]. This antigen, though not fully characterized chemically, appeared to be a phosphoglycolipid sensitive to phospholipase and is present in the lipid extracts of *Plasmodium falciparum* infected erythrocytes. It was reported that the antigen is mainly synthesized late in schizogony and is also shed from the infected erythrocytes. Amount of GPL obtained by us from 1-liter culture supernatant is very little (10µg) and it is plausible that this may also be shed by the infected erythrocytes. The specificity and immunoreactivity of the GPL observed here are not surprising if it contains GPL identified by Mabs earlier. Further, our GPL is well conserved among different isolates. We, for the first time, report on this aspect and demonstrate the excellent diagnostic potential of GPL antigen for detect and demonstrate the excellent diagnostic potential of GPL antigen for detection of *Plasmodium falciparum* infection.

The remarkable specificity and sensitivity of GPL in LIA for *Plasmodium falciparum* is demonstrated cent percent correlation with the slide examination results (Table 1). The assay is based on antibody detection, it

cannot be recommended for clinical diagnosis but it can be used as an excellent epidemiological tool. Surveillance and control of malaria require evaluation of Annual parasite index (API), Slide positive rate (SPR) or Slide *Plasmodium falciparum* positive rate (SPFR), which are often under reported due to human tardiness and errors. Also evaluations of these parameters are highly laborious and time intensive. In such situation, GPL/LIA assay described here can be useful for supplementing or cross checking SPR data or determining *Plasmodium falciparum* transmission dynamics in endemic areas.

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**Tumour necrosis factor-alpha (TNF- α), interleukin-10 (IL-10)
and interferongamma (IFN- γ) in leprosy**

**Khin Saw Aye, *Yin Thet Nu Oo, *Ei Mon Thet, *Kyi Kyi Aye,
**Kyaw Kyaw & **Tin Mg Tsoh*

**Immunology Research Division
Department of Medical Research (Lower Myanmar)
**Yangon General Hospital*

Leprosy is a chronic infectious disease characterized by a broad spectrum of clinical forms depending on the patient's immune response, in particular cell-mediated immune response. Cytokines can play a role in the cell-mediated immune response. Serum levels of Interferon-gamma (IFN- γ), Interleukin-10 (IL-10), Tumour Necrosis Factor (TNF- α), were measured by enzyme-linked immunosorbent assay (ELISA) in 45 leprosy patients with reaction and 30 age and sex matched healthy controls. Significantly higher serum levels of the cytokines were detected in leprosy patients compared to controls. Serum cytokine levels of paucibacillary (PB) patients have significantly higher level of IFN- γ and TNF- α than those of multibacillary (MB) patients. MB patients have significantly higher serum level of IL-10 than PB patients. Patients with type I reaction have high levels of IFN- γ and those of type II reaction have increased level of IL-10. In leprosy patients, IFN- γ and TNF- α are immunoprotective and IL-10 is immunosuppressive. Results indicate that type I reaction is a cell mediated immune response and type II reaction is essentially an immunocomplex disease.

INTRODUCTION

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, which is an obligated intracellular pathogen [1]. It is characterized by a broad spectrum of clinical forms depending on the host's immune response, in particular cell-mediated immune (CMI) response, which is responsible for the defense against intracellular pathogen [2]. Patients exhibit a strong CMI response to *M. leprae* at the tuberculoid pole and it declines toward the lepromatous pole where patients exhibit a defective CMI response to *M. leprae* [3].

Cytokines act as molecular signals for communication between cells of immune system and play a role in CMI response of leprosy patients. Cytokine dysregulation is responsible for selective defect in the CMI response to *M. leprae* in lepromatous patients [4, 5]. Skin lesions of MB patients

showed an increased production of the suppressive T-helper 2 (TH₂) cytokines (IL-4, IL-5 and IL-10) [6], a decreased production of the protective T-helper 1 (TH₁) cytokines (IL-2 and IFN- γ) and macrophage cytokines (TNF- α) [4, 6].

Acute episodes "reaction" may occur during the chronic course of leprosy. The reactional states are classified as either type I (reversal reaction) or type II (erythema nodosum leprosum, ENL) [7]. The former is attributed to a rapid change in the CMI response [8] and the latter to immune complex pathogenesis [9]. However, CMI response has been shown to play a role in its pathogenesis [10]. Cytokines, especially the proinflammatory cytokines (TNF- α), may be involved in reactional states of leprosy [11, 12]. *In vivo* and *in vitro* studies are used to delineate the immunologic aspects of

leprosy and its reactional states. *In vivo* studies are better tools to detect what actually occur in patients and one of them is the measurement of serum cytokines produced by cells of the immune system in response to *M. leprae*. It is a simple and less expensive method compared to other *in vivo* approaches e.g. immunohistochemical and polymerase chain reaction (PCR) studies. The aim of the study is to delineate the serum cytokine levels of leprosy patients with reaction.

MATERIALS AND METHODS

Forty-five leprosy patients (25 PB and 20 MB) classified according to WHO criteria [14] from the Central Special Skin Clinic (CSSC), Yangon General Hospital and 30 age and sex matched healthy controls from National Blood Bank were studied. Of these cases, 36 patients have reactions (20 type I and 16 type II).

Detailed history taking, medical and dermatological examination, slit-skin smear for bacteriologic and morphologic indices and serum samples were collected from all patients. Serum samples were aliquoted into 0.5 ml volume and stored at -80°C until tested. TNF- α , IL-10 and IFN- γ cytokine levels were measured by enzyme-link immunosorbent assay (ELISA) (TECHNE Corporation, Minneapolis, U.S.A) with the lower detection levels of 10pg/ml, 5pg/ml and 100pg/ml, respectively. The data were analyzed using SPSS software. Student's *t*-test was used to compare the patients and the control group.

RESULTS

All patients have significantly high serum cytokine levels compared to controls MB patients have high IL-10 ($p<0.001$) and low IFN- γ and TNF- α ($p<0.001$) compared to PB patients (Table 1). Patients with type I reaction have high IFN- γ ($p<0.001$)

(Table 2) and those of type II reaction high IFN- γ and IL-10 ($p<0.05$) (Table 3).

Table 1. Serum cytokine levels of leprosy patients and control

(pg/ml)	Control	PB	MB	Whole group
IFN- γ	148.00 $\pm$ 31.81	1015.63 $\pm$ 227.12*	460.67 $\pm$ 254.57*	707.31 $\pm$ 367.87*
IL-10	32.05 $\pm$ 7.65	55.58 $\pm$ 17.65*	92.45 $\pm$ 23.72*	78.87 $\pm$ 30.82*
TNF- α	13.15 $\pm$ 4.02	76.66 $\pm$ 52.16*	31.35 $\pm$ 20.72*	50.12 $\pm$ 43.72*

* $p<0.001$

PB = paucibacillary

MB=multibacillary

Table 2. Serum cytokine levels of PB with and without Type I reaction

(pg/ml)	PB	PB with Type I reaction	<i>P</i>
IFN- γ	833.7 $\pm$ 279.46	1260 $\pm$ 191.4	***
IL-10	70.46 $\pm$ 23.16	66.67 $\pm$ 9.82	NS
TNF- α	54.67 $\pm$ 46.25	34.86 $\pm$ 24.51	NS

*** $p<0.001$

PB = paucibacillary NS = not significant

Table 3. Serum cytokine levels MB with and without Type II reaction

(pg/ml)	MB	MB with Type II reaction	<i>P</i>
IFN- γ	346.00 $\pm$ 180.0	562.50 $\pm$ 173.68	*
IL-10	94.5 $\pm$ 4.94	111.0 $\pm$ 21.07	*
TNF- α	32.10 $\pm$ 20.67	35.00 $\pm$ 14.14	NS

* $p<0.05$

MB = multibacillary

NS = not significant

DISCUSSION

Cell-mediated immunity is responsible for defense against intracellular pathogens, such as *M. leprae*. TH₁ cells are activated at the tuberculoid pole resulting in strong cell-mediated immunity whereas activated TH₂ cells in turn inhibit TH₁ cells at the lepromatous pole leading to defective cell-mediated immunity [18].

All patients have significantly higher serum cytokine levels compared to controls indicating stimulation of cells of the immune system by *M. leprae* antigens even in MB patients with known T-cell unresponsiveness to *M. leprae* [19].

PB patients have significantly higher serum IFN- γ and TNF- α levels compared to MB patients indicating activation of TH₁ cells which are the major source of IFN- γ [20]. T-cells from PB patients stimulated with *M. leprae* produced high levels of IFN- γ compared to that of MB patients [21, 22]. In addition, it has been shown that skin lesion of PB patients showed significantly high IFN- γ production compared to MB patients [4-6]. IFN- γ plays a central role in protection against intracellular bacteria [23]. The protective value of IFN- γ in leprosy was evident from the reduction of viable *M. leprae* organisms at the site of intradermal injection of IFN- γ [24, 25].

When the two types of leprosy reaction were compared, type I patients had significantly elevated levels of IFN- γ while type II patients had significantly elevated levels of IL-10. These findings could explain the difference in the pathogenesis of the two types. Type I reaction is a CMI reaction and the role of IFN- γ in cell-mediated immunity is well known [26]. Type II reaction is essentially an immune complex disease and the role of IL-10 in augmenting humoral immune responses is well documented [27].

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**Potential source of infection through vegetables
with a note on bacterial pathogens isolated from children with diarrhoea**

**Mar Mar Nyein, *Mi Mi Htwe, **Khin Myat Tun & **Kyi Kyi Aye*

*Bacteriology Research Division
**Clinical Research Division
Department of Medical Research (LM)

Distribution of bacterial pathogens was studied on 350 specimens of vegetables from bazaars and salads from street vendors in 10 townships in three seasons during March 2000 to August 2001. Similarly, 62 cases of diarrhoeic children attended the Yangon Children's Hospital during May to August, 2001 were tested for isolation of pathogens. All the vegetables and salads were heavily contaminated with coliforms and faecal coliforms. *Salmonella* species, *Shigella dysenteriae* and *S. sonnei* were isolated from Thaketa, Insein and Mingaladon respectively. *Vibrio cholerae* was isolated from Tamwe and Kamayut. *S. dysenteriae* was isolated from coriander (nan-nan-pin) and *V. cholerae* from hsala. Various serogroups of *Escherichia coli* (O6K15, O28a/cK73, O25K+, O86K62, O142K+, O146K89, O148K+) were isolated from vegetables: *Aeromonas hydrophilia*, enteropathogenic *E. coli* (O27K+, O28a/cK73, O111K58, O114K90, O119K69, O128K67, O148K+, O157K+, O159K+), *Plesiomonas shigelloides*, *S. dysenteriae* A, *S. sonnei* phase 1, *V. cholerae* O1 (Ogawa) and *V. cholerae* O139 were isolated from 27 cases (43.55%) of diarrhoeic children. Studies on antibiotic resistance and plasmid patterns reveal that some clinical and environmental *E. coli* possess similar pattern of resistance gene. Thus, environmental health care is important to eliminate transmission of infections. From experiments, just washing with clean water 11 times could not eliminate faecal coliforms totally. However, washing vegetables at least three times with clean water (1 litre each for 50 grams) and treating with 0.001 percent of potassium permanagate for 30 minutes and again washing out with clean water for three times could eliminate faecal coliforms.

INTRODUCTION

Environmental risk factors play an important role on health in many countries. Children are exposed to a range of health risks within their environment and in many cases they play, or work, in environments that are detrimental to health. Contaminated water and food increase the risk of enteric infections including cholera, shigella and salmonella. Young children are at high risk of exposure because they are usually with their mothers around the cooking area. Every year about 11 million children die

before they reach their fifth birthday. Of these, about 8 million children die from no more than five infections including diarrhoeal diseases [1]. As World Health Organization has estimated that up to 70 percent of diarrhoeal diseases may be due to contaminated food and water, these vehicles may be responsible for the majority of the reported illness annually and a great number more unreported [2]. This research, thus, aims to find out transmission of enteric pathogens through vegetables, pasteurized Yangon Children's Hospital during 2000 to milk and diarrhoeic children attended 2001.

MATERIALS AND METHODS

Study period

March 2000 to September 2001.

Transportation

Vegetables in plastic bags, milk as it is cooled and rectal swabs from children in Cary Blair medium through cold chain were transported within 30 minutes to three hours from collected area to the laboratory.

Sample size

A total of 320 specimens of vegetables, (gazun, kyethunmeik, khayanthi, gobidouk, nannanpin, pinzein, pusinan, mounlaouhpyu, mounlapin, myinkhwa, shaukywet, thayetkin and thakhwathi) from 10 township bazaars (Pabedan, Ahlone, Insein, Tamwe, Mingala-don, S. Okkalapa, Kamayut Thaketa, Sanchaung and Yankin) were tested in three seasons. Also, salads (tosaya) from 30 street vendors (htaminsaing) which were located in those townships were included in this study. At least five to 10 kinds of varieties were prepared as tosaya either in fresh, boiled or pickled forms in each preparation. Also, 62 cases of diarrhoeic children attended at Yangon Childrens Hospital were studied for isolation of enteric pathogens.

Determination of coliforms and faecal coliforms

Presumptive test by multiple tube technique was done by using MacConkey Broth Purple using single and double strength and the presence of coliforms and faecal coliforms was determined by incubating at 37°C and 44°C respectively. Confirmation was done by using Brilliant Green Bile medium with their corresponding presumptive positive tube using respective temperatures [3].

Determination of Total Bacterial Count

Dilutions were done by sterile phosphate buffer if necessary and 0.02 ml of each dilutions was placed onto Nutrient agar

plates, allowed to dry at room temperature and incubated at 37°C [4].

Determination of enteric bacterial pathogens

Approximately 20 ml of samples were spinned and the residue was used for inoculation onto Blood agar, MacConkey agar, Salmonella-Shigella agar, Mannitol Salt agar, Thiosulphate Citrate Bile Sucrose agar for primary isolation. Simultaneously, enrichment media of Selenite F and Alkaline Peptone water were used for secondary inoculation. The suspected colonies were then picked from respective media and were confirmed biochemically and serologically [5].

RESULTS

Environmental studies from vegetables in different townships

As shown in Table 1, all the tested vegetables in three seasons from 10 townships were contaminated with coliforms and faecal coliforms. *Shigella dysenteriae* and *S. sonnei* were isolated from Insein and Mingaladon respectively in October, whereas, *Vibrio cholerae* was isolated from Tamwe in August and Kamayut in November respectively. Moreover, *Salmonella* species was isolated from Thaketa in August and October.

Isolation of bacterial pathogens from children attended the Yangon Children's Hospital during 2001

As shown in Table 2, bacterial pathogens were isolated from 27 cases. These include *Aeromonas hydrophila* (3.23%), *Escherichia coli* (EPEC) (19.36%), *Plesiomonas shigelloides* (1.61%), *Shigella dysenteriae* A (3.23%), *Shigella sonnei* phase1 (4.84%), *Vibrio cholerae* O1 (8.07%), and *Vibrio cholerae* O139 (3.23%). *A. hydrophila*, *P. shigelloides*, *S. sonnei* and *V. cholerae* O1 were isolated from 4 cases (44.44%) of the age group of <6 months. From age group of 6-12 months, *S. dysenteriae*, *S. sonnei*, *V. cholerae* O139, *E. coli* EPEC (O28acK73,

O114K90, O128K67 & O159K+) were isolated from 9 cases (45%). From age group of 13-24 months, *A. hydrophila*, *E. coli* (O27K+, O119K69, O148K+, O157K+ & O159K+) were isolated. From the age group of 25-36 months, *S. dysenteriae*, *S. sonnei* and *V. cholerae* O1 were isolated. *V. cholerae* was isolated from age group of 37-48 and 48-60 months (Table 3).

Association of bacterial pathogens isolated from stool samples and from vegetables in different townships

As shown in Table 4, it was noted that isolation of same species had occurred in Insein and Mingaladon with *S. dysenteriae*

and *S. sonnei* phase 1 from stool samples and vegetables respectively.

Total bacterial count in different types of vegetables after washing

A total of 14 different kinds of vegetables weighing 2.2 to 140.4 grams in each case due to the availability of the products were washed with two litres of clean water (free from coliforms and faecal coliforms) up to eleven times. Total bacterial count, coliform and faecal coliform counts were performed by Miles and Misra method. The count obtained after washing is shown in Table 5.

Table 1. Bacterial pathogens isolated from vegetables in different places in three seasons

Place	Hot & Hot wet 7-3-00 to 11-7-00			Wet 18-7-00 to 16-9-00			Cool 21-9-00 to 4-12-00		
Papedan	1	7-3-00	C +FC	12	18-7-00	O119K69 C +FC	22	21-9-00	O28a/c C +FC
Ahlone	2	13-3-00	C +FC	13	25-7-00	C +FC	23	2-10-00	O25K+ O86K62 O142K+ O148K+ C +FC
Insein	3	21-3-00	O126K71 C +FC	14	31-7-00	O28a/c O111K58 C +FC	24	9-10-00	<i>S. dys.</i> C +FC
Tamwe	5	2-5-00	C +FC	15	8-8-00	O28a/c O111K58 O125K+ <i>V. c.</i> (O) C +FC	28	13-11-00	C +FC
Mingaladon	6	31-5-00	O126K71 C +FC	16	15-8-00	O86K61 O111K58 O142K+ C +FC	25	16-10-00	<i>S. son.</i> C +FC
S.Okkalapa	7	6-6-00	C +FC	17	21-8-00	C +FC	26	23-10-00	C +FC
Kamayut	8	13-6-00	C +FC	19	3-9-00	O111K58 O119K69 C +FC	29	21-11-00	O146K89 <i>V. c.</i> (O) C +FC
Thaketa	9	20-6-00	O26K60 O86K62 C +FC	18	28-8-00	O111K58 O142K+ O148K+ <i>Salmo.</i> C +FC	27	30-10-00	O6K15 O146K69 <i>Salmo.</i> C +FC
Sanchaung	10	4-7-00	C +FC	20	11-9-00	O111K58 O28a/c C +FC	30	27-11-00	<i>S. dys.</i> C +FC
Yankin	11	11-7-00	C +FC	21	16-9-00	O28a/c C +FC	31	4-12-00	C +FC

C = Coliforms serogroups
S. dys. = *Shigella dysenteriae*
Salmo. = *Salmonella* species

FC = Faecal coliforms

EPEC = Enteropathogenic *Escherichia coli*
S. son. = *Shigella sonnei*
V. c.(O) = *Vibrio cholerae* (Ogawa)

Table 2. Bacterial pathogens isolated from children with diarrhoea (62 cases) attended the Yangon Children's Hospital during June-August 2001

Pathogens isolated	Males n=34	%	Females n=28	%	Total
<i>A. hydrophila</i>	2	5.88	0	0	2 (3.23)
<i>E. coli</i> (EPEC)	10	29.41	2	8.00	12 (19.36)
<i>P. shigelloides</i>	1	2.94	0	0	1 (1.61)
<i>S. dysenteriae</i> A	1	2.94	1	3.57	2 (3.23)
<i>S. sonnei</i> phase 1	2	5.88	1	3.57	3 (4.84)
<i>V. cholerae</i> O1	3	8.82	2	7.14	5 (8.07)
<i>V. O139</i>	1	2.94	1	3.57	2 (3.23)
Total	20	58.82	7	25.00	27 (43.55)

Figures in parenthesis denote percentages

It was found that the count decreased to 36 organisms/ml in lemon leaves after washing four times. The radish plants which were smeared with soil in roots still contained uncountable number of bacteria even after washing up to 11 times. However, these bacteria were not detected after dipping the vegetables in 0.001% potassium

permanganate for 30 minutes and washed again three times with clean water.

DISCUSSION

A food-borne disease outbreak (FBDO) is defined as an incident in which two or more persons experience a similar illness resulting

from the ingestion of a common food. In Myanmar, diarrhoea, dysentery, food poisoning, typhoid and paratyphoid fevers are at the top list of Diseases Under Surveillance (Notifiable Diseases). These are all food-borne diseases and WHO had shown that each year some 1500 million episodes of diarrhoea occur in children under the age of five and over three million die as a direct result. It has been estimated that up to 70 percent of diarrhoeal diseases may be caused by contaminated food. This also includes the water used in the preparation of food, washing of hands, food and utensils [7].

Table 3. Distribution of bacterial pathogens from different age groups of children attended the Yangon Children's Hospital during 2001

Age group (months)	Tested males	Pathogens isolated	Tested females	Pathogens isolated	Total
<6	6	<i>A. hydrophila</i> <i>P. shigelloides</i> <i>S. sonnei</i> <i>V. cholerae</i> O1 = 4 cases	3	nil	4 / 9 (44.44)
6-12	9	<i>S. dysenteriae</i> <i>V. cholerae</i> 0139 0114 K 90 0128 K 67 0159 K + = 5 cases	11	<i>S. sonnei</i> <i>V. cholerae</i> 0139 028a/ck73 0114K90 = 4 cases	9/20 (45)
13-24	13	<i>A. hydrophila</i> 027 K + 0119 K 69 0148 K + 0157K + 0159 K + = 6cases	11	<i>V. dysenteriae</i> 01 027K+ 0157K+ = 3 cases	9/24 (37.50)
25-36	5	<i>S. sonnei</i> <i>V. cholerae</i> 01 = 2 cases	2	<i>S. dysenteriae</i> A = 1 case	3/5 (60)
37-48	0		1	<i>V. cholerae</i> 01 = 1 case	1/1
48-60	1	<i>V. cholerae</i> 01 = 1 case	0		1/1
Total	34	18 (52.94)	28	9 (32.14)	27/62(43.55)

Figures in parenthesis denote percentages

Table 4. Association of bacterial pathogens isolated from stool samples and from vegetables in different townships

Sr. No.	Townships	Cases	Pathogens isolated from	
			Diarrhoeic children	Vegetables
1	Ahlone	5	<i>E. coli</i> O159K+*	<i>E. coli</i> O25K+, O86K62, O142K+, O148K+
2	Dagon (North)	2	<i>A. hydrophila</i> <i>V. cholerae</i> O1	Not tested
3	Insein	4	<i>S. dysenteriae</i> <i>V. cholerae</i> O1	<i>E. coli</i> O28 a/c, O111K58, O126K71 <i>S. dysenteriae</i>
4	Kamayut	5		<i>E. coli</i> O111 K58, O119K69, O146K89 <i>V. cholerae</i> 1
5	Kyauktan	3	<i>S. sonnei</i> (2 cases) <i>V. cholerae</i> O139	Not tested
6	Mingaladon	4	<i>E. coli</i> O157 K+ <i>S. sonnei</i>	<i>E. coli</i> O126K71, O142K+ <i>S. sonnei</i>
7	Papedan	4	<i>E. coli</i> O157K+; O159K+* <i>V. cholerae</i> O1	<i>E. coli</i> O28a/cK73, O119K69
8	Sanchaung	3	<i>E. coli</i> O128K67 <i>V. cholerae</i> O1	<i>E. coli</i> O28a/cK73 O111K58
9	Tamwe	7	<i>E. coli</i> O27K+; O28a/cK73; O148K+* <i>S. dysenteriae</i> A <i>V. cholerae</i> O139	<i>E. coli</i> O28 a/cK73 O111K58 O125K+ <i>V. cholerae</i> O1
10	Thaketa	13	<i>E. coli</i> O27K+; O119K69 <i>P. shigelloides</i> <i>V. cholerae</i> O1	<i>E. coli</i> O6K15 O26K15, O86K62, O146K69 <i>Salmonella</i> spp.
11	Thingangyun	6	<i>E. coli</i> O114K90 *(2 cases) <i>A. hydrophila</i>	
12	Others	6	nil	nil
	Total	62	27 cases	31 samples

Table 5. Total bacterial count in different vegetables after washing with 2 litres of clean water (initial count= uncountable organisms/ml or >1800MPN/100ml)

Myanmar	English	Scientific name	Weight (grams)	Washing times	Count or g/ml
1. Gazun	water convolvulus	<i>Ipomoea aquatica</i>	101.8	8	350
2. Kyethunmeik	onion tops	<i>Allium cepa</i>	49.1	7	375
3. Khayanthi	egg plant	<i>Solanum melongena</i>	80.2	6	400
4. Gobidouk	cabbage	<i>Brassica oleracea</i>	140.4	6	150
5. Hsalat	lettuce	<i>Lactuca sativa</i>	9.7	8	40
6. Nannanpin	coriander	<i>Coriandum sativum</i>	42	11	150
7. Pinzein	basil	<i>Ocimum sanctum</i>	31.3	6	250
8. Pusinan	mint	<i>Mentha arvensis</i>	15	8	280
9. Mounlaouhpyu	radish		78	11	515
10. Mounlapin	radish plant		32	11	UC
11. Myinkhwa	pennywort	<i>Hydrocotyl asiatica</i>	32.2	6	250
12. Shaukyet	lemon	<i>Citrus hystrix</i>	16.2	6	36
13. Thayetkin		unknown	2.2	9	400
14. Thakhwathi	Cucumber	<i>Cucumis sativus</i>	92.6	6	350

UC = Uncountable

MDN = Most Probable Number

Access to good quality, safe and nutritious food is considered as basic right of the people. Consumption of unsafe, contaminated food leads to food-borne diseases which cause considerable morbidity and mortality. In many countries, the diseases transmitted by food are commonly referred to as food poisoning and are characterized by abrupt onset of gastrointestinal disturbances viz. abdominal pain, vomiting and diarrhoea. The foods most commonly involved in food-borne disease are meat and meat products, poultry, eggs, milk and milk products, sweet meats and rice preparations. In Myanmar, cholera is the most frequent food-borne outbreak. Every year there are pockets of endemic or epidemic outbreaks occurring throughout the country. The main cause is due to contamination of food during preparation or storage before serving. Incidence of infections caused by some pathogens viz. *Bacillus cereus*, *Staphylococcus aureus*, *Clostridium perfringens* is probably very high, however, as the disease are often self limiting, they receive little attention to public health services, unless there is mass involvement. Typhoid and paratyphoid food-borne outbreak is also common [7]. Our data, mainly concised on Enterobacteriaceae and Vibrionaceae, had shown that organisms from these two families were isolated from all the three sources. It revealed that potential source of infection among diarrhoea cases of children came from foods which were available easily in their environment. Thus, environmental sanitation and personal hygiene still play important roles to eradicate infections especially among young children.

The recommendations from these findings are:

1. Do not eat vegetables without washing.
2. As it may be difficult to make free of pathogens from vegetables, children

should not be allowed to touch things before washing to be cooked.

3. Do not believe that all milk are well pasteurized even labelling.
4. Eat fresh things (just after cooking) as much as possible.
5. Do not eat leftover dishes especially after keeping for more than 6 hours.
6. Fresh yogurt is most suitable for consumption.
7. Always prepare for good sanitation.

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**Incidence, case fatality rate and treatment - seeking behaviour
of the snakebite victims from two townships of Myanmar**

Tun Pe, *Aye Aye Myint, **Sunn Htut, *Sann Mya,
****Khin Aye Kyu & ****Maung Maung Toe*

**Venom Research Laboratory
Department of Medical Research (Lower Myanmar)
**Kyaukpadaung Civil Hospital
***Department of Medicine, Institute of Medicine (2), Yangon
****Department of Railway, Taungdwingyi
*****Department of Medical Research (Lower Myanmar)*

A community-based study of incidence, case fatality rate and treatment-seeking behaviour of snakebite victims was carried out in Taungdwingyi (TDG) and Kyaukpadaung (KPD) townships. A house-to-house visit was conducted by an assigned midwife and a structured questionnaire was asked to the victims or next of kin if the victim was dead. The incidence of snakebite for 4 yrs (1999-2002) is 1381 (746TDG +635KPD) with yearly incidence increases from 29 to 100/100000 in KPD and in TDG from 76 to 115/100000 (1999 to 2001) and 72/100000 in 2002. The average case fatality rate remains high in both townships (12.9%KPD and 19.3%TDG). Age specific morbidity and mortality is highest in age group 21-30yrs in TDG. High morbidity and mortality of snakebites is observed during ploughing and harvesting seasons. The snakes responsible for most bites are Russell's viper, cobra and green pit viper. Occasional bites by king cobra, kraits and nonpoisonous snakes were recalled. Conjunctivitis following spitting of venom into eyes by a spitting cobra in TDG was also recalled. Majority of them were farmers and bitten on lower limbs (79%) while engaged in farming. No longer recommended wound treatments are still in practice. Majority (64%) of the victims sought treatment from traditional healers in KPD and that of TDG at hospital (79%). Among the hospital admissions, primary cases had a better prognosis compared to secondary and no hospital admissions in TDG ($p < 0.001$). Pre hospital antivenom was given in 31% of the victims from TDG. In spite of health education, only a few (25/1381) wore boots as a prophylaxis. The high mortality and morbidity rates of snakebite could be attributed to lack of use of prophylaxis against snakebite, incorrect treatment seeking behaviour and attitude of the victims, which call for further implementation of the health education programme.

INTRODUCTION

Snakebite occurs throughout 14 States and Divisions of Myanmar and yearly incidence based on hospital data from the whole country collected by the Department of Health Planning [1] accounts for 7710 (6529-8994). There is a decreasing trend of incidence from 1998 to 2000 (7684 to 6529) and upward trend from 2001 to 2003 (7877

to 8994). The case fatality rate varies between years (1998-2003) from 4.9% to 8.5% (mean 7.1%). Snakebite occurs throughout the year in some localities with the introduction of multiple cropping. However, Russell's viper bite occurs during harvesting and ploughing seasons [2,3]. It is an occupational hazard of farmers and plantation workers. Although many species of poisonous snakes are around, hospital

records show bites by three common species of snake namely Russell's viper (*Daboia russelii siamensis*), cobra (*Naja kaouthia*) and green pit vipers [4,5]. Bites by many important species are not on hospital record but bites by king cobra (*Ophiophagus hannah*) [6], Chinese krait (*Bungarus multicinctus*) [7], common krait (*Bungarus fasciatus*) [7], Malayan pit viper (*Calloselasma rhodostoma*) [8,9], and venom ophthalmia following spitting venom into eyes by spitting cobra (*Naja mandalayensis*) [10] have been reported earlier. It appears that there is under reporting of snakebite cases as well as bites by important species of snake. A community-based study of epidemiology of snakebite may reveal true prevalence of snakebite and has been carried out in some countries [11-13]. It is high time that a community-based study of epidemiology of snakebite and treatment-seeking behaviour of the snakebite victims needs to be conducted in order to reveal the true incidence and magnitude of the problem. Such study was conducted in two townships, Taungdwingyi (TDG) and Kyaukpadaung (KPD) where snakebite is endemic.

MATERIALS AND METHODS

A community-based survey of incidence, case fatality rate and treatment seeking behaviour of the victims was carried out in Taungdwingyi (TDG) and Kyaukpadaung (KPD) townships. A house-to-house survey was carried out by an assigned midwife from February to May 2002 in TDG and from February to June 2003 in KPD. A structured questionnaire designed to cover circumstances of the bite, mortality, sequelae, treatment seeking behaviour, use of first aid and prophylaxis was asked to the victims or next of kin if the victim was dead covering a period of 4 years (1999-2002). For children, guardian or parents were asked. Coded data were entered and analysed using Epi info version (6.04D) software. Statistical analysis was carried out

using student "t" test. $P < 0.05$ was considered as significant.

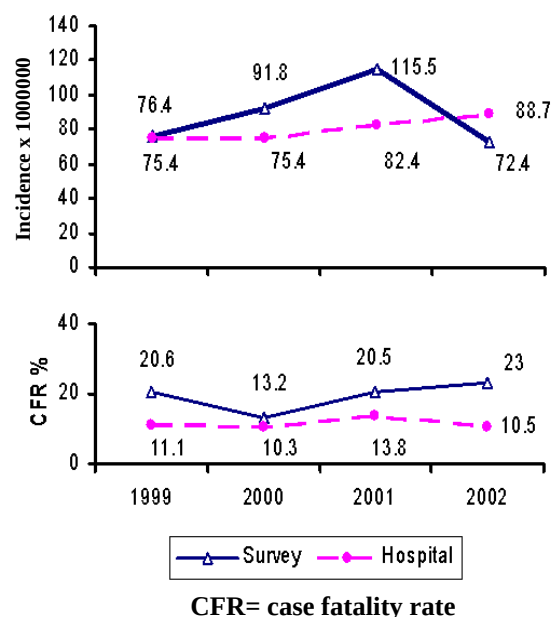


Fig. 1. Incidence and case fatality rate of snakebite cases of Taungdwingyi

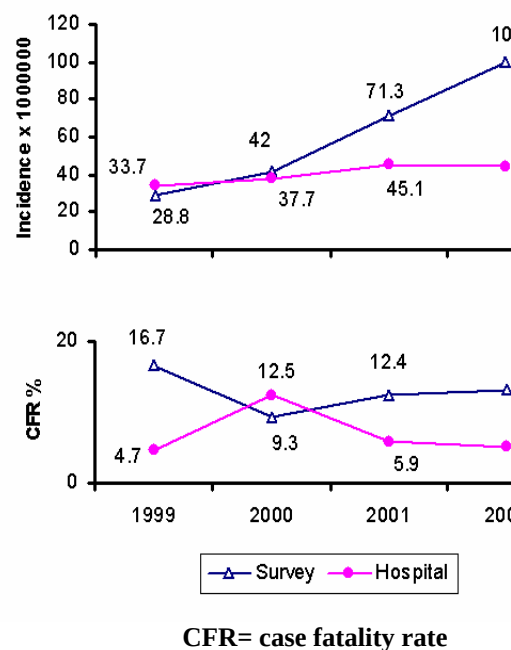


Fig. 2. Incidence and case fatality rate of snakebite cases of Kyaukpadaung

RESULTS

Incidence and mortality

A house-to-house visit by an assigned midwife was conducted on a total

population of 480020 (215312) Taung-dwingyi and 264708 Kyaukpadaung) residing in two townships. The total number of snakebite recalled by the victim in 4 years (1999-2002) is 1381 (746, TDG and 635, KPD). The yearly incidence of snakebite from 1999 to 2002 in TDG increases from 76.4 to 115.5/100000 (1999-2001) and 72.4/100000 in 2002 (Figure 1) and that of KPD is 28.8 to 100.1/100000 (Figure 2). Snakebite occurs throughout the year with peak incidence at ploughing (May) and harvesting (October) seasons in KPD and throughout the year in TDG (data not shown).

The age of the victim ranges from 3- 84 yrs (mean age 30yr) and 79% are less than 40 yrs old. Male to female ratio is 1.8:1 (895:486). Majority 1093 (79.9%) of the victims were farmers and the rest included 75 (5.5%) teachers, students and monks, 72 (5.3%) off-hand workers, 67(4.9%) street-vendors, 28 (2%) palm workers, 28 (2%) dependents and 5 (0.4%) livestock-breeders. Forty three percent (594) of the victims were alone at the time of the bites and 57% (787) had company. Majority (79%) (1090) of the bites occurred during the day (6am - 5pm) and 21% (291) after dark (6pm-5am).

The case fatality rate of snakebite in TDG is 19.3% (13.2-23%) and that of KPD 12.9% (9.3-16.7%). The overall case fatality rate due to Russell's viper bite is 18% (21% TDG and 14% in KPD) and cobra 11% (16.6%TDG and 8.8% KPD). TDG has high fatality rate following Russell's viper (21%) and cobra (16.6%) bites compared to KPD. Four out of 7 King cobra bites are fatal.

The age specific morbidity and mortality (excluding 71 and above age groups) is highest in age group 21-30 yr in TDG and varies between years in KPD.

The snakes

Of total 1363 bites, Russell's viper (*D.r.siamensis*) accounts for 1122 (82%), followed by cobra (*N. kaouthia*) 104 (8%) and green pit viper 77 (6%). Cobra bite is more common in KPD (14%). King cobra

(*Ophio. hannah*) bite causes 4/7 death in TDG. Chinese krait (*B. multicinctus*) bite accounts for 5 in KPD and 2 in TDG with no fatality. Non-fatal banded krait (*B. fasciatus*) bite, one in each township was recalled. One victim suffered from venom ophthalmia following spitting of venom into eyes by a spitting cobra (*N. mandalayensis*) was recalled in TDG. Nonpoisonous bite of 16 *Hamalopsis buccata*, 8 *Ptyas mucosus* and one *Chrysopelia ornata* were also recalled (Table 1).

Site of bite

Majority of the victims were bitten on lower limb 78.3% (n=1381) and 20.9% (n=1381) on upper limb. Bites on unusual sites: 6 at buttock (three while reaping grass, on way to toilet, while preparing jaggery paste and a fatal bite on buttock of a 3-year-old girl by a Russell's viper), on eye-lid while handling a nonpoisonous snake, on head by a Russell's viper while asleep and on ear and forehead by green pit vipers were recalled. Venom ophthalmia following spitting of venom into eyes by a spitting cobra while minding livestock was recalled.

Prophylaxis and first- aid

About 46% (n=1492) of the victims used protective wear at work (the victims from KPD (71%) used it twice more than TDG 36%). However, majority (92.4%) were still wearing rubber sandals and only 17 wore local/imported boots and 8 DMR fang-proof boots (in TDG). Majority (99.9%) of non protective wear users did not use any protective measure against snakebite.

Of 17 local/imported boot users from TDG, 7/9 were envenomed and 3 died. Remaining two (one received pre hospital antivenom) and all eight fang-proof boot users did not develop signs of envenoming.

Wound treatment was carried out in 83% of the victims. Tourniquet (52%) was widely applied by the victims followed by wound incision (12.7%), application of herbal medicine to wound (10.9%) and padding of chicken breast to wound (2%). Traditional

Table 1. Snake species responsible for the bites

Activity	Russell's viper	Cobra	Green pit viper	King cobra	CK	BK	SC	H.b	Pt.mu	Ch.o	Uk	Total
Farming	584(87%)	42(6%)	26(4%)	2	3			6		1	11	675
Minding livestock	122(84%)	16(11%)	4(3%)				1		1		2	146
Looking for food*	142(84%)	2	18(11%)	3		1		1			3	170
Way to video house	178(76%)	30(13%)	13(6%)	1	2	1			3		7	235
Collecting fire wood	31(76%)		6(15%)	1				1			2	41
Domestic work	50(72%)	7(10%)	7(10%)		2			1	2			69
Palm worker	14(58%)	7(29%)	3(13%)									24
Handle snake	1								2			3
Total	1122(82%)	104(8%)	77(6%)	7	7	2	1	9	8	1	25	1363

* Catching frogs, rats, birds, snakes and plugging vegetables

CK= Chinese

SC=Spitting cobra krait

H.b=*Hamalopsis buccata*

BK= Banded krait

Pt.mu = *Ptyas mucosus*

Ch.o=*Chrysopelia ornata*

belief of swallowing snake's tail accounts for 1.5%. Practice of harmful wound incision (23.3%), tattooing (6%) and application of herbal extract to the site of bite (14.4%) were more common in KPD and application of chicken pad to the site of bite (2.9%) and swallowing of snake's tail (2.6%) in TDG. Only 17% of the victims from TDG applied local compression immobilisation first aid technique and in contrast only 4/635 (0.7%) victims applied the first-aid in KPD.

Circumstances of bite

Majority 686 (50%) were bitten while at farming. Some were bitten on way to video house and visiting friends (18%). More people were bitten while looking for food (26.5%) and minding live stock (12.7%) in TDG compared to KPD. Bites within house premises (4%) in TDG and while doing domestic work (6.1%) in KPD account for few instances. Bite among toddy palm workers accounts for 3.8% in KPD. Three were bitten while handling non poisonous snakes.

The snakes responsible for most bites in all circumstances are Russell's viper (82%), cobra (8%) and green pit viper (6%) (Table 1). Cobra bite was frequent in people visiting video house and friends and while minding livestock. King cobra,

Chinese krait and banded krait bites were encountered while at farming, looking for food, visiting video house and friends, among toddy palm workers and while minding livestock. Venom ophthalmia following spitting of venom into eyes by a spitting cobra while minding livestock was recalled.

Treatment seeking behaviour

Treatment seeking behaviour of the victims of two townships differs from each other (Table 2). Majority 63.6% of the victims from KPD compared to 15.6% in TDG prefer local healers' treatments. About twice the number of victims from TDG (79.2%) compared to KPD (43.3%) sought hospital treatment and at rural health centre and clinic. About three times more victims from KPD (54%) prefer to remain in villages after taking local treatment. Primary and secondary hospital admission seekers in KPD are about two times less than that of TDG. It is interesting to note that all victims [24] from Sanmyint village of KPD sought treatment at Duwon monastery.

Majority of the victim (55%) sought hospital treatment soon after the bite in TDG compared to 27% in KPD and 70 to 88% within 4hr after the bite. Traditional healers' treatments consist of either ingestion of herbal extracts or local application of

Table 2. Treatment seeking behaviour of the snakebite victims

	Taung-dwingyi	Kyauk-padaung	Total(%)
Treatment sought	451(60%) n 745	517(81%) n 635	968 (70)
Traditional healer	116 (15.6%)	329 (63.7%)	445(46.5)
Rural health centre	239 (53%)	140 (27.1%)	379(39.6)
Unspecified	53 (7.1%)	2	55 (5.7)
Monastery		24 (4.6%)	24 (2.5)
Clinic	16 (2.1%)	6 (1%)	22 (2.3)
Traditional clinic	13 (2.9%)	12 (2.3%)	15 (1.6)
Voluntary health worker	10 (1.3%)		10 (1.0)
Other hospitals	1	4 (0.7%)	5 (0.5)
Home treatment	1		1 (0.1)
Hospital treatment	590 (79.2%) n 745	275 (43.3%) n 635	865 (62.7)
Primary	283 (38%)	102 (16.1%)	385 (27.9)
Secondary referrals	307 (41.2%)	173 (27.2%)	480(34.8)
No hosp. adms.	142 (19%)	344 (54.2%)	486 (35.2)
No treatment	13 (1.8%)	16 (2.5%)	29 (2.1)

meditated edible oil or herbal extract or wound incision and suction.

Fatality among hospital and non-hospital admissions

There is a significant difference in case fatality rate between primary hospital admissions 10.6% (n=283), secondary referrals 15.9% (n=307) and non-hospital admissions 42.2% (n=154) in TDG ($P<0.001$). A similar difference is observed between primary 8.8% (n=102) and secondary referrals 23.1% (n=173) of KPD ($p<0.001$), however no difference is observed between primary 8.8% (n=102) and non hospital admissions 7.3% (n=344) ($p=0.63$)

Wound treatment on prognosis of hospital admissions

Although first-aid/wound treatment practiced by the victims are not ideal, there is a difference in fatality rate among primary admissions with or without wound treatment ($p<0.05$) and that of secondary referrals ($p<0.05$) in TDG and in secondary

referrals of KPD ($P<0.05$), however, no difference in fatality rate is observed among primary admissions with or without wound treatment of KPD ($p=0.7$).

Symptoms following the bites

Majority (75%) of the victims developed local and systemic symptoms following the bites, however these symptoms are more marked in cases from KPD (Table 3).

Table 3. Symptoms recalled by the snakebite victims

	Taungdwingyi	Kyaukpadaung	Total (%)
Systemic symptoms			
Developed in	564(75%) n 746	116(75%) n 154	680(75)
Oliguria	132(23.4%)	48(41.4%)	180(26.5)
Haematemesis	45(7.9%)	34(29.3%)	79(11.6)
Shock	44(7.8%)	32(27.6%)	76(11.2)
Haematuria	37(6.6%)	37(31.9%)	74(10.8)
Renal failure	25(4.4%)	13(11.2%)	38(5.58)
Haemoptysis	21(3.7%)	19(16.4%)	40(5.88)
Local features			
Developed in	584(78%) n 746	485(77%) n 634	1069(77)
Pain	488(83.6%) n584	362(57.1%)	850(62)
Swelling	428(75%)	376(59.3%)	804(58)
Blister	62(10.6%)	13(2.1%)	75(5.4)
Ulcer	57(9.7%)	12(1.9%)	69(5.0)

History of previous snakebite was recalled in 9% of the victims. The common species of snake responsible for most bites were Russell's viper, cobra and green pit viper.

Only 11% of the victims from KPD and 31% from TDG recalled receiving prehospital antivenom in villages. Majority (55%) of the victims killed the inflicted snakes for identification.

Knowledge on use of prophylaxis and first-aid

In general, the victims acquired health information from basic health workers (57.6%), family/village elders (16.1%), television educational programme (15.9%) and educational pamphlets (10.4%). Forty

three percent of the victims have knowledge on use of prophylactic fang-proof boots of DMR against snakebite and 80% of the victims from TDG and 27% of KPD know local compression (with cotton pad) and immobilisation first-aid used in snakebite.

Majority (77%) of the victims from KPD and 42% from TDG recalled acquiring health education on prophylaxis and first-aid in snakebite from basic health workers and 25% in TDG and 5% in KPD from family/village elders and 24% in TDG and 6% in KPD from television educational programme and 9% in TDG and 12% in KPD from health educational pamphlets. Majority (55%) of the victims saw the snakes before the accident. Forty four percent of the victims believed that all snakes were poisonous.

DISCUSSION

Incidence

Epidemiological study of snakebite based on hospital returns in early 1970 and 1980 [14] shows that incidence of snakebite in Magwe Division drops from 46.1/100000 (1970) to 20.4/100000 (1980) and in Mandalay Division it remains fairly stable (35.5 to 35.1/100000) for respective years. Recent epidemiological study of snakebite based on 87 hospital statistics of six divisions of Myanmar (1998-2000) [4] also shows decrease in average yearly incidence of snakebite (37.2 to 20.2/100000) in Magwe and 38.26 to 29.05/100000 in Mandalay Division. It is supported by hospital-based data collected from the whole country by the Department of Health Planning [1] that yearly incidence of poisonous snakebite decreases from 1998 to 1999 in Magwe and Mandalay divisions. However, its incidence increases from year 2000 to 2003. The present community-based studies of snakebite carried out in Taungdwingyi (Magwe Division) and Kyaukpadaung (Mandalay Division) (1998-2002) also show a similar rising trend of incidence of snakebite.

An earlier study carried out at Taungdwingyi hospital in 1994 [3] shows a high incidence of snakebite (126.1/100000) with a mortality rate of 8.95%. However, the present community-based study (1999-2002) shows that the yearly incidence of snakebite in Taungdwingyi (Magwe Division) is on increase from 76.4 to 115.5/100000 for (1999-2001) and decreases to 72.4/100000 in 2002. However, a similar rising trend is observed in Kyaukpadaung (Mandalay) (29 to 100/100000). Results indicate that incidence of snakebite in two townships is on increase in contrast to earlier observations [4]. The yearly incidence of snakebite recalled by the victims from TDG and KPD (1999 to 2002) compared to that of respective hospital statistics (Figure 1 & 2) are high, suggesting that the true incidence of snakebite is grossly underestimated.

Increase in incidence of snakebite may be due to increased contact between human and snake with the introduction of multiple cropping as well as failure of taking prophylactic measures against snake bite at work. The rising trend of incidence of snakebite calls for review of effectiveness of snakebite control programme and needs for implementation of effective health education since snakebite is a preventable disease. Decrease in incidence of snakebite in TDG in 2002 (although hospital records show increasing trend of snakebite) could be attributed to population movement or increased number of snakebite cases coming from neighbouring townships seeking treatment at TDG in that year.

Case fatality rate

The case fatality rate of snakebite for the whole country based on hospital data [14] drops from 6.7% (1981) to 3.9% (1988). Recent hospital-based study (1998-2000) [4] also shows decrease in the rate from 5.8% to 3.75%. Case fatality rate of snakebite in Magwe (1998-2000) drops from 13.4% to 5.2% and 5% to 3.8% in Mandalay. The present community-based study (1999-2002) shows that the rate remains

high in Taungdwingyi 19% (range 13.25% to 23%) and 13% (9.3% to 16.7%) in Kyauk-padaung. Moreover, it is observed that the case fatality rate of hospital statistics (1998-2000) is about 50% less than the survey data, suggesting that the true case fatality rate is also grossly underestimated.

The high fatality rate of the victims could be attributed to failure of using prophylaxis against snakebite and correct first-aid, incorrect treatment seeking behaviour of the victims and late referrals.

Snakebite is an occupational hazard of our farmers where majority are bitten during ploughing and harvesting seasons with high morbidity and mortality. Age specific morbidity and fatality is highest in age group of 21-30 yr in TDG, which represents the working force of the country. Similar observations were made in our earlier studies on snakebite cases admitted to township hospitals [5].

The snakes

The study confirms that Russell's viper, cobra and green pit viper are responsible for most bites [2, 3, 5]. Venom ophthalmia following spitting of venom into eyes by spitting cobra (*N. mandalayensis*), King cobra and krait bites recalled in the survey were not recorded in hospital data but similar accident/bites occurring in townships through enquiry have been reported earlier by our groups [6,7,10,15]. The study highlights that there are many unreported bites by important species of snake and hospital data grossly underestimates the true incidence of snakebite.

Majority of king cobra bites are fatal because of severe envenoming. However, it is emphasized that lives of neurotoxic envenomed cases could be saved by providing artificial or assisted ventilation in the absence of specific antivenom [6, 15].

Circumstances of the bites

Snakebite is an occupational hazard of farmers and most (60%) are bitten while engaged in farming and others in various

circumstances. Since all living in the community are at risk, it is suggested that all-out wearing of prophylactic boots will safeguard the community against snakebite [16].

The study confirms the earlier observation made on Russell's viper bite cases [2, 3, & 5] that legs (79%) and upper limbs (21%) are common sites of bite. Bites on ear, forehead and buttock are uncommon.

First-aid

Applying tourniquet is still widely practiced among the victims in spite of its ill effects [17]. Wound incision ± application of herbal extract, widely practiced in KPD, is prone to get infection and bleeding from the wound in systemic envenomed Russell's viper bite cases. Swallowing snake's tails and application of chicken breast to wound have no scientific value. Such harmful and unscientific practices should be discouraged. Since only a few victims applied local compression immobilisation first-aid, it calls for review of the effectiveness of dissemination of the health education programmes to the community.

Treatment seeking behaviour

The number of victims (31%) receiving pre hospital antivenom in TDG is doubled since our last study in 1994 [3], however it accounts for only 11% in KPD. It has been shown that early administration of adequate dose of antivenom soon after the bite in village carries a better prognosis and less complication [18]. A large-scale study on feasibility and efficacy of early adequate dose of antivenom given in villages should be made.

The study highlights that majority of the victims from TDG are health conscious and two times more victims from TDG compared to KPD have perception on seeking early treatment at hospital. In contrast, majority (63.6%) of the victims of KPD believed in local healers' treatments suggesting that traditional belief has much influence on treatment seeking behaviour of the victims of KPD. It is supported by the

fact that 54% of the victims after receiving local treatment remain at home and numbers of primary/secondary hospital admission seekers are two times less than that of TDG. Wrong treatment seeking in KPD resulted in development of more systemic cases with complications (data based on symptoms recalled 30% vs. 10%) compared to TDG. It is likely that traditional belief rather than accessibility to health centres is responsible for high morbidity in KPD.

It is high time that health education leading to behavioural change in concept on treatment seeking behaviour including seeking early medical treatment at hospital, discouraging unscientific traditional healers' treatments and home remedy which give a false sense of security should be aimed at in imparting health education to the communities.

It is observed that primary hospital admissions carry a better prognosis compared to secondary referrals and no admissions. However, in KPD no significant difference in prognosis is observed between primary admissions and no hospital admissions probably the latter received mild envenoming compared to that of TDG (reflected in case fatality rate of 7.3% and 42.2% respectively).

Moreover, wound treatments practiced in KPD (wound incision $\pm$ application of herbal extract to wound) do not influence the prognosis of primary admission in contrast to that of TDG. The study highlights that victims should be encouraged to seek early hospital admission and unscientific treatment of local healers should be discouraged. The study reveals that pattern of treatment seeking behaviour and perception of the victim influence the outcome of the victim. Health education should be directed toward achieving change in attitude of the community in treatment seeking behaviour.

Knowledge

Although majority of the victims acquired knowledge on snakebite prophylaxis and the

first-aid from basic health workers (77% in KPD and 42% in TDG), in practice, only a few cases used it. It is high time that health education leading to behavioural change should be aimed at in future dissemination of health information on prevention and use of correct first-aid in snakebite.

Efficacy of fang-proof boots

The study highlights that none (8/8) of fang-proof boots (innovated by Department of Medical Research, Lower Myanmar) users were envenomed further supporting fang proof efficacy of DMR (LM) boots [16]. Two local/imported boot users were probably not envenomed or may receive trifle envenoming that was neutralised by early antivenom in one. It has been observed that rubber or canvas materials could not withstand penetration of Russell's viper fangs (personal observation).

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Seroepidemiology on extended contacts of new leprosy cases in Nyaungdon Township

**Khin Nwe Oo, *Nwe Nwe Yin, *Tin Tin Han & **Kyaw Myint*

**Immunology Research Division*

Department of Medical Research (Lower Myanmar)

***Leprosy Control Programme, Department of Health*

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

INTRODUCTION

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

In Myanmar, leprosy elimination was achieved at national level at the end of January 2003. Prevalence rate of 70 townships mainly from Ayeyawady, Bago, Mandalay, Sagaing Divisions and Shan State is higher than elimination level. Up to June 2003, New Case Detection Rate (NCDR) is more than 4/10,000 in 6 hyperendemic divisions and southern Shan State. NCDRs of Ayeyawady and Bago Divisions are higher than other 4 hyperendemic divisions in 2002 as well as in first half of 2003 [2].

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

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

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

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

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

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

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

MATERIALS AND METHODS

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

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

NTP-BSA ELISA technique

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

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

RESULTS

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

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

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

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

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

PB = Paucibacillary type of leprosy

MB = Multibacillary type of leprosy

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

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

PBHC = Household contacts of paucibacillary leprosy

PBNC = Neighbor contacts of paucibacillary leprosy

MBHC = Household contacts of multibacillary leprosy

MBNC = Neighbor contacts of multibacillary leprosy

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

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

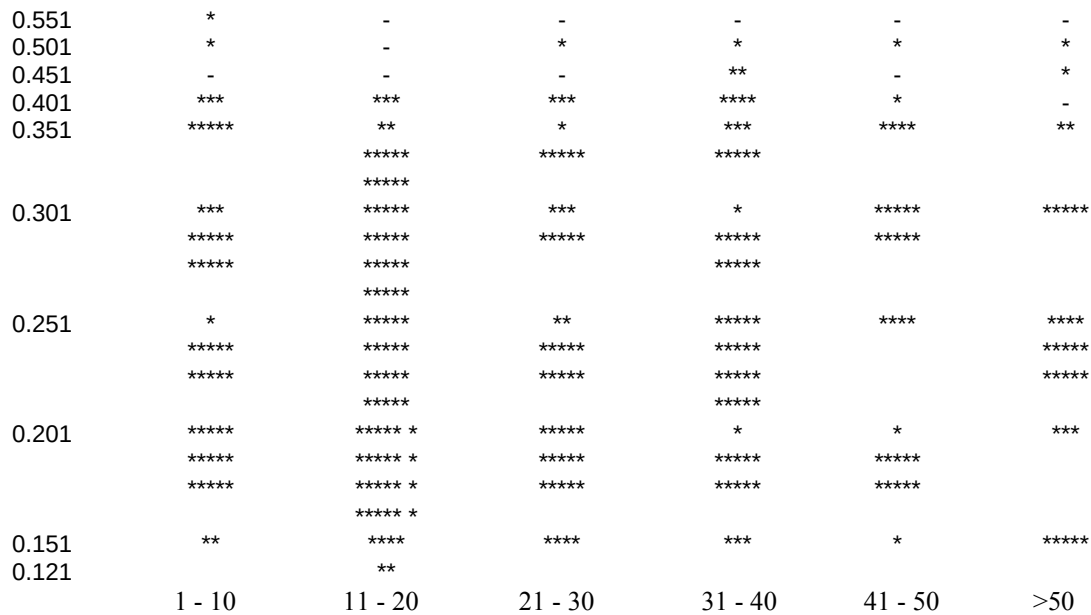


Fig. 1. Scattergram showing seropositives among extended contacts of new cases of leprosy by age range in Nyaungdon Township
Cut-off point = 0.120 (absorbance at λ 492 nm)

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

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

PBHC =Household contacts of paucibacillary leprosy

PBNC=Neighbor contacts of paucibacillary leprosy

MBHC =Household contacts of multibacillary leprosy

MBNC =Neighbor contacts of multibacillary leprosy

IgM index = Mean anti IgM antibody titers /cut-off value

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

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

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

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

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

DISCUSSION

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

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

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

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

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

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

The extended contacts having highly increased antibodies were present in both

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

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Cost-effectiveness analysis of contraceptive options in Central Women's Hospital

*Khin Htay, **Than Than Tin & ***Phone Myint

*Central Women's Hospital

**University of Medicine (1)

***Department of Health Planning

The aim of this study is to determine the most cost-effective method of contraception in Central Women's Hospital. Based on the cost data obtained from clients attending Central Women's Hospital during the study period, costs for 1000 hypothetical clients for each method for a period of 3 years were estimated. After reviewing literatures, using estimated failure rates from literature (Trussell *et al.*, 1995), number of unintended pregnancy avoided for one year were calculated. It was found that IUCD was the most cost-effective. It was followed by the injection depoprovera and sterilization. Combined oral contraceptives were the least cost-effective method. This study explores the most cost-effective method in currently used contraceptives and it may be useful in implementing family planning services cost-effectively.

INTRODUCTION

Contraceptive technology is a medical success story. For women who should not become pregnant because of medical problems, contraception saves lives and prevents morbidity. For the majority of users, contraception enhances quality of life, allowing couples to choose when they wish to have children [1].

The two irreversible methods, female surgical sterilization and male surgical sterilization (vasectomy), provide the greatest protection from unintended pregnancy. Reversible methods of contraception are oral contraceptives, the injectable contraceptives, subdermal implant, intrauterine devices, barrier methods, fertility awareness methods, lactational amenorrhoea method and coitus interruptus [2]. Cost-effectiveness analysis is a form of economic evaluation. It is one facet of information in choosing an appropriate method of contraception [3].

MATERIALS AND METHODS

This study was a methodological study, where a method for evaluating cost-

effectiveness of contraceptive options is developed using prospective approach. It was conducted in family planning clinic, Central Women's Hospital from March 2002 to May 2003. During the study period, 30 clients for each method of contraception i.e., combined oral contraceptives, injectable depo-provera, copper-T IUD and female sterilization were interviewed.

Costs of different methods

Costs incurred by the users only were considered. During the period of study, individual clients were asked, using the proforma, about the costs incurred for drugs and travel costs. For oral contraceptives and injectables, costs incurred during the period of 9 months were recorded. Costs that would be incurred for one year period were then estimated. For the remaining two methods, cost incurred at the time of investigation was taken as one time cost for the particular year as the method of (IUCD) is used for long term (3-5 years), and sterilization has permanent effect.

Costs were calculated for 1000 hypothetical clients using each method for 3 consecutive years.

Costs of each method include two parts, cost for those using the method throughout the year and costs for those using only to the point where failure takes place.

$$\begin{aligned}
 \text{Users at the beginning} &= U \\
 \text{Drop out rate} &= \text{Users at the beginning} \times \text{failure} \\
 &= U \times f \\
 \text{Users at the end of the year} &= U - Uf \\
 &= U(1-f) \\
 \text{Costs for those using the method throughout the year} \\
 \text{(total users) will then be,} &= U(1-f) \times C \\
 &= UC(1-f) \quad [4]
 \end{aligned}$$

Costs for those partially using the method

Costs for those who fail to use the method throughout the year include costs up to the time they fail to use the method only.

For those using pills or injectables, assumption was made that all of them drop out at mid point of the year. They use the method for six months only.

$$\begin{aligned}
 \text{Number of partial users} &= \text{Initial users} \times \text{failure rate} \\
 &= U \times f \\
 \text{Cost for partial users} &= Uf \times \frac{1}{2}C \quad [4] \\
 \text{Total costs} &= \text{Cost for total users} + \\
 &\quad \text{costs for partial users} \\
 &= UC(1-f) + \frac{1}{2}UCf \\
 &= C(U - \frac{1}{2}Uf) - (1) \quad [4]
 \end{aligned}$$

For calculating costs for subsequent years the equation (1) can be extended as follows:

$$\text{Cost for the second year} = C(U - Uf_1)(1 - \frac{1}{2}f_1)$$

$$\text{Cost for the third year} = C[(U - Uf_1)(1 - \frac{1}{2}f_2)(1 - \frac{1}{2}f_3)]$$

For IUCD method, assumption was made that drop out took place at the end of the year and costs for removal of IUCD were included in calculating total costs of using IUCD for one year.

If the cost for removal is C_r and the number of drop out is Uf as previously calculated.

$$\begin{aligned}
 \text{Removal costs} &= Uf \times C_r \text{ and,} \\
 \text{Total costs} &= \text{Costs for total users} + \\
 &\quad \text{costs for removal} \\
 &= UC + UfC_r \\
 &= U(C + fC_r) - (2) \quad [4]
 \end{aligned}$$

Annual failure rate for the method available in one literature reviewed [4] were applied in this study.

As the period under consideration was three years, costs were discounted for annual rate of change in price of drugs and appliances, which was assumed to be 10%. Sensitivity analysis was done and changing annual discount rate did not affect the conclusion.

Effectiveness of each method of contraception

Effectiveness of each method is determined as the number of unintended pregnancy avoided [4].

$$U_{pav} = N(C_0 - f_1)$$

$$\begin{aligned}
 U_{pav} &= \text{Unintended pregnancy avoided} \\
 C_0 &= \text{Contraception rate without any method} \\
 f_1 &= \text{failure rate of particular method} \\
 N &= \text{Number of user}
 \end{aligned}$$

Cost-effectiveness of each method

Cost-effectiveness of each method is expressed as cost per unintended pregnancy avoided [4].

RESULTS

Table 1. Costs undiscounted for annual rate of change in price for each contraceptive method used by 1000 hypothetical clients in 3 years

Method	Costs (Kyats)		
	Year (1)	Year (2)	Year (3)
COC	2993120	2903326	2816226
Injectable depo-provera	2290789	2283916	2755248
Copper-T IUD	1131330	262.66	787.98
Female sterilization	8282000	-	-

Table 2. Costs discounted for annual rate of change in price for each contraceptive method used by 1000 hypothetical clients in 3 years

Method	Cost (Kyats)			Total discounted cost in 3 years (Kyats)
	Year (1)	Year (2)	Year (3)	
COC	2993120	3193659	3407633.5	9594412.1
Injectables	2290789	2512308	2755248.7	7558345.3
Copper-T IUD	1131330	288.93	593.5	1133623.1
Female sterilization	8282000	-	-	8282000

Table 3. Pregnancy avoided

Method	Year	User	Pregnancy expected in one client		Pregnancy avoided
			Without	With	
COC	1	1000	0.85	0.03	$1000 \times 0.82 = 820$
	2	970	0.85	0.03	$970 \times 0.82 = 796$
	3	940	0.85	0.03	$940 \times 0.82 = 772$
	Total	-	-	-	2388
Injectables	1	1000	0.85	0.003	$1000 \times 0.847 = 847$
	2	997	0.85	0.003	$997 \times 0.847 = 845$
	3	994	0.85	0.003	$994 \times 0.847 = 842$
	Total	-	-	-	2534
IUCD	1	1000	0.85	0.008	$1000 \times 0.842 = 842$
	2	992	0.85	0.002	$992 \times 0.848 = 842$
	3	990	0.85	0.006	$990 \times 0.844 = 836$
	Total	-	-	-	2520
Sterilization	1	1000	0.85	0.004	$1000 \times 0.846 = 846$
	2	996	0.85	0.0013	$996 \times 0.8487 = 845$
	3	994	0.85	0.0013	$994 \times 0.8487 = 842$
	Total	-	-	-	2537

Table 4. Cost-effectiveness of different methods

Method	Total costs (Kyats)	Total pregnancy avoided	Cost/pregnancy avoided
COC	9594412.06	2388	4017.76
Injectables	7558345.25	2532	2982.77
IUCD	1133623.03	2520	449.85
Sterilization	8282000	2537	3264.4

The methods under study can be listed on the basis of cost-effectiveness as follows:

- (1) Copper-T IUD
- (2) Injectable depo-provera
- (3) Female sterilization
- (4) Combined oral contraceptives

DISCUSSION

This study showed that copper-T IUCD was the most cost-effective, costing 449.85 Kyats for each unintended pregnancy avoided. Injectable depo-provera was the second most cost-effective, costing 2982.77 Kyats. Female sterilization was the third most effective, 3264.4 Kyats. Oral contra-

ceptives were the least cost-effective as they cost 4017.76 Kyats for each pregnancy avoided.

Trussell *et al.*, (1995) had been reported that copper-T IUD was the most cost-effective method.

In this study, the result was the same because copper-T IUD is cheap, easily available, has less side effect and lower failure rate.

Trussell *et al.* (1995) showed that Injection depo-provera was the fourth most cost-effective method next to copper-T IUCD, vasectomy and implant.

In this study Injection depo-provera was the second most cost-effective method behind copper-T IUCD, because most of the clients use this method, and also due to other reasons. It is suitable for breastfeeding mothers, clients with difficulty in remembering to take a pill and painful periods. It has less side effect and low failure rate. It is also easily available in our country.

Mathematica Policy Research (1992) showed that tubal ligation was the second most cost-effective method behind vasectomy. Trussell *et al.*, (1995) found that tubal ligation was the seventh most cost-effective method behind copper-T IUD, vasectomy, implant, injectable, oral contraceptives and progesterone - T IUD.

In this study, tubal ligation was the third most cost-effective method following copper-T IUD and injection depo-provera. Although failure rate for tubal ligation was the lowest, the highest costs incurred, so the method was found to be less cost-effective than IUCD and injectables.

Trussell *et al.*, (1995) had reported that oral contraceptives were the fifth most cost-effective method following copper-T IUD, implant and injectable contraceptive.

In this study, oral contraceptives were the least cost-effective method following copper-T IUD, injectables and sterilization. Contraceptive pills were not free from costs

and the costs were more or less comparable to those injectable but because of high failure rate it was found to be least cost-effective.

Mathematica Policy Research (1992) showed that vasectomy was the most cost-effective method. Trussell *et.al.*, (1995) found that vasectomy was second most cost-effective method next to copper-T IUD. In our country, vasectomy is not legally available.

Mathematica Policy Research (1992) showed that Norplant was the third most cost-effective method. Trussell *et al.*, (1995) also found that Norplant was the third most cost-effective method. In our country, Norplant is not available now.

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**Proportional physical disabilities of women following child births
in rural areas of delta region in Myanmar**

Thein Thein Htay, **Than Tun Sein, *Khin Myint Myint Than, ***Yin Yin Soe,
Mon Mon, *Theingi Myint, **Ko Ko Zaw, *Khin Mar Myint,
*Hla Mya Thwe Einder & **Myo Myo Mon*

*Department of Health
**Socio-medical Research Division
Department of Medical Research (LM)
*** University of Medicine 2, Yangon

A community-based survey was conducted in 5 delta townships to explore the situation of obstetrical physical disability in Myanmar. Three hundred and thirty three ever-married women of 15-59 years age group who have had at least one normal full term delivery were examined for birth related disability conditions and face-to-face interviews made. Forty three of them were again interviewed in-depth. Interviews were also made with 35 key informants. The findings indicated: prevalence of urethrocoele (22%), cystocoele (83%), cervical descent (22%), rectocoele (32%), perineal tear (32%) and enterocoele (14%); urethrocoele, cervical descent, rectocoele and enterocoele were significantly more prevalent among 30-44 age group ($p<0.01$); the conditions, except enterocoele (having association with 5-10 years of schooling; $p<0.05$), had no significant associations with education and social status of mothers; only 13% of the women sought for care for the conditions; about 55% of their deliveries were made with traditional birth attendants or other untrained accouchers; and this birth attendant utilization had no statistically significant associations with age, education or social status of the women. There were only 2 women suffering from vesico-vaginal fistulae. Our study findings call for attention to be paid to various kinds of physical disabilities that Myanmar rural women are suffering following births; and also for consideration to impart TBAs with safe and clean delivery skills.

INTRODUCTION

Complications of pregnancy and childbirth are the leading causes of disability and death among women between the ages of 15-49 [1]. Over 18 million women each year endure chronic disabilities as a result of their pregnancy. Perineal trauma at the time of delivery can result in dyspareunia and perineal pain. Urinary incontinence, anal incontinence and uterovaginal prolapse are the major long term pelvic floor sequelae of childbirth [2]. Other consequences include chronic pelvic sepsis and pain, recurrent pelvic inflammatory disease, and infertility [3]. Pregnancy and labour is also associated with orthopaedic complications such as

separation of pubis symphysis, transient osteoporosis of the hip and osteonecrosis of the femoral neck which can cause serious disability [4]. Apart from these physical disabilities, childbirth is also associated with psychiatric problems most commonly maternity blues, postnatal depression and puerperal psychosis [5]. Moreover, their potential productivity is diminished, primarily due to death, malaria, anaemia and the consequences of obstructed labour - fistula and urinary incontinence [6]. Many of the maternal disabilities also jeopardize the survival and health of newborn infants.

In Myanmar there is virtually no community-based study on obstetrical

physical disability. Obstetrical physical disability arises out of poor socio-economic conditions and the physical disability that arises will further perpetuate low socio-economic status due to unemployment, chronic life difficulties, and poor health. Analyzing and understanding the situation of obstetrical physical disabilities among women can provide policy inputs for formulation of health policies for enhancing quality of maternal and child health care in rural areas of Myanmar.

This study was conducted with the objectives of identifying physical disability conditions following child birth among rural mothers and analyzing the socio-economic and demographic factors relating to the disability conditions.

METHODOLOGY

Study type

The study was a community-based survey using both quantitative and qualitative methods. Quantitative data was meant for comparing disability situation according to women's socio-economic and demographic factors. Data collection depended on both self-reported morbidity and observed morbidity. Qualitative data provided in-depth information on contextual factors relating to the disability situation.

Sample and sampling procedures

The quantitative component recruited 361 ever-married women who have had at least one normal full term delivery either vaginally or by Caesarean section. They were of 15-59 years age group with difficulties in performing usual activities following childbirths. Women who were pregnant or who were seriously ill to participate in interviews were excluded.

This quantitative component used a modified EPI cluster sampling method: 30 clusters (village tracts) were randomly selected, and at least 14 eligible women

(participants for the survey) were selected randomly in each cluster.

Ayeyawady Division was chosen purposively. Ayeyawady Division is a delta area where transport and communication have markedly improved during the past decade. Five townships - Patheingyi, Hinthada, Wakema, Dedaya and Maubin - were randomly selected from the list of townships located in the Division.

In each township, three Rural Health Centers (RHCs) were selected randomly. The village where the RHC is stationed was taken as one cluster. Another cluster was chosen randomly among the villages where a RHC Sub-center is located in the jurisdiction of each of the RHCs. This made six clusters to be included in each township and thus gave thirty clusters for the whole study.

A list of women of 15-59 years age group meeting the criteria set was prepared with the local informants. Selection of at least 14 women was made from the list randomly in each cluster. The recruited mothers were examined for birth related disability conditions and were also interviewed face-to-face. Although it was expected to recruit 420 mothers, only 361 who met the set criteria could be recruited; among them, only 333 samples with acceptable quality were used for data analysis.

The qualitative component involved performing in-depth interviews (IDIs) with 6 mothers having physical disabilities following childbirths, and Key Informant Interviews (KIIs) with 1 TBA, 1 AMW and 1 Lady Health Visitor/Midwife in selected clusters. The Research Team decided the clusters where the qualitative component was to be included after arrival to the study township. There were 43 IDIs and 35 KIIs for the whole study.

Data analysis

The statistical package of STATA8.1 was used for all analyses. We used Pearson chi-

square to assess significant differences in the prevalence of child delivery related disabilities among rural women of different age, educational and occupational groups. Qualitative data were analyzed using Atlas ti software.

During face-to-face interviews, eighteen questions with measurement scales ranging from 1-6 were asked to each of the sampled women to assess impact of their current health problem(s) on certain aspects of life. These health domains included: overall satisfaction with life; moods and feelings; sense of confidence; ability to wash oneself, use toilet, dress oneself, feed oneself, assist other members of one's family, sit or stand, carry things (e.g., a bucket of water), use a vehicle (e.g., a bus), move around and within one's house, move around one's neighbourhood; ability to relate to people in authority (e.g., government officials), neighbours, friends and relatives; ability to participate in family activities (e.g., eating together) and in community activities.

Each health domain (variable) was given a score of 1-6 according to expressions made by the interviewees. Thus for the total of all the 18 variables, the minimum score is 18 and the maximum score is 108. This range is subdivided into 3 categories of scores: score 18 for having no impact at all; scores 19-36 for having some impacts; and scores 37 and above for those with extreme impacts.

RESULTS

Quantitative findings

Characteristics of study samples

Fifty one percent of the study population came from the age group 30-44 years, 52% had had 5-10 years schooling and 58% were married at ages between 13-20 years (see Table 1). In rural areas, poor families are generally referred to as *let-loke-let-sar*. They are manual labourers who do not have a stable income most of the time. In our study, we used the two broad categories - manual labourers (representing *let-loke-let-*

sar) and others (i.e., relatively well-to-dos) - to identify occupational groups of our rural population. Table 1 indicates that only about a third of our study population falls into manual labourers.

Table 1. Characteristics of 333 women in Ayeyawady Division in 2004

Characteristics	No. of women ^a
Age (years)	
15-29	78 (23)
30-44	168 (50)
45-59	87 (26)
Education (years of schooling)	
0-4	128 (38)
5-10	175 (53)
11-15	30 (9)
Age at first marriage (years)^b	
13-20	192 (58)
21-38	139 (42)
Job category^c	
Manual labourers (<i>let-loke-let-sar</i>)	89 (28)
Others (relatively well-to-dos)	230 (72)

^a Values in parentheses are percentages; due to rounding of the numbers, they may not total 100.

^b The total number for this measure was 331. The total number for this measure was 319.

Prevalence of child delivery related disabilities

Cystocoele (83%) was found to be highly prevalent among the 333 subjects included in the study. Other obstetric related disability conditions included urethrocoele (22%), cervical descent (22%), rectocoele (32%), enterocoele (14%) and perineal tear (32%). Sixty per cent and 51% of the cystocoeles and rectocoeles fell into moderate to large grades respectively; and 73% of the perineal tears were of either second or third degrees.

The four disability conditions (urethrocoele, cervical descent, rectocoele and enterocoele) were found to be more prevalent among 30-44 age group, and the distribution was statistically significant ($p < 0.05$). Except for the enterocoele having statistically

Table 2. Percentage of disability conditions detected on vaginal examination of 333 women, according to different age, educational and occupational characteristics, in Ayeyawady Division in 2004

Women's characteristic	Disability condition present (%)					
	Cystocoele (N=277)	Urethrocoele* (N=74)*	Cervical descent** (N=213)	Rectocoele** (N=105)	Perineal tear (N=310)	Enterocoele (N=46)*
Age group (years)						
15-29	24	36	29	35	25	28
30-44	51	47	51	49	50	61
45-59	25	16	20	16	25	11
	Cystocoele (N=277)	Urethrocoele (N=73)	Cervical descent (N=212)	Rectocoele (N=104)	Perineal tear (N=309)	Enterocoele* (N=46)
Educational group (years of schooling)						
0-4	36	30	35	36	37	26
5-10	53	58	56	52	54	57
11-15	10	12	9	11	9	17
	Cystocoele (N=276)	Urethrocoele (N=73)	Cervical descent (N=211)	Rectocoele (N=103)	Perineal tear (N=308)	Enterocoele (N=46)
Occupational group						
Manual labourer (N=96)	29	26	28	27	30	26
Others (N=236)	71	74	72	73	70	74

* Distributions of a disability condition between different sub-groups of women's characteristics significantly different at $p < 0.05$; **significantly different at $p < 0.01$. Percentages may not total 100 because of rounding.

significant association ($p < 0.05$) with education of women, the other disability conditions have no statistically significant associations with the education and occupation of the subjects studied. See Table 2.

There were 2 study subjects having vesico-vaginal fistulae. Detailed descriptions of these two cases are described in later part of this report.

Seeking care for disability conditions

Among these women with child delivery related disability conditions, only 13% sought care for the conditions they were suffering. Seeking care has no statistically significant associations with women's age, education and occupation.

Impact of the disability conditions on daily activities

About 76% of the interviewees said that their disability conditions were having some impacts on their daily activities. See Table 3 for the results.

Table 3. Scores of 331 women for impact on certain aspects of life due to disability condition and distress that ensues, in Ayeyawady Division in 2004

Scores	Women getting the scores ^a
<i>Impact scores</i>	
18 (no impact)	61 (18)
19-36 (some impact)	250 (76)
37+ (severe impact)	20 (6)

^a The total number for this measure was 276; values in parentheses are percentages

Except for statistically significant association between expressed impact and education ($p < 0.05$), age and occupational group have no statistically significant relationships to the impact on daily activities (see Table 4).

Birth attendants

The women included in our study had experienced a total of 1469 deliveries. Out of these deliveries, 55% were made by traditional birth attendants (TBAs); 25% by midwives and Lady Health Visitors; 10% by auxiliary midwives; and 10% at hospitals.

Table 4. Percentage of 331 women in the three categories of impact scores, according to different age, educational and occupational characteristics, in Ayeyawady Division in 2004

Women's characteristic	Women getting impact scores (%)		
	18 no impact (N=61)	19-36 some impact (N=250)	37+ severe impact (N=20)
Age group (years)			
15-29	28	23	15
30-44	51	50	60
45-59	21	27	25
Educational group (years of schooling)*			
0-4	43	38	35
5-10	39	55	60
11-15	18	7	5
Occupational group			
Manual labourer	25	31	15
Others	75	69	85

*Distributions of impact scores between different sub-groups of women's characteristics significantly different at $p < 0.05$

Most of the deliveries (88%) took place at homes; 10% at hospitals; and 2% at health centers. This birth attendant utilization has no significant associations with age, education or occupation of the mothers.

Qualitative findings

Health staff claimed that local situations have changed in birth attendant utilization patterns; local mothers were said to be utilizing trained staff (midwives) more. However, traditional birth attendants (TBAs) said they were the key persons who were making deliveries. The reasons for continued popularity of TBAs included: local poor mothers could not afford to make their deliveries with midwives; TBAs were always easily accessible in times of deliveries; TBAs stayed with mothers throughout the puerperial period, performing some household chores for the mothers. However, TBAs admitted that for the first child, local mothers preferred

delivering with a midwife; and in times of difficult labours, a TBA and a midwife performed the delivery together.

Answers given by mothers were also more or less reflect the information received from health staff and TBAs.

"The TBA stays closer to us ... she is easily accessible"

(A 50-year old woman of 4 children, with cervical descent)

"Delivering with TBAs is our mi-yoe-pha-lar (traditional practices of ancestors) ... All my children were delivered by a TBA"

(A 31-year old woman of 2 children, with cervical descent)

"It is safe to deliver with a midwife. All my children were delivered with a midwife"

(A 49-year old woman of 4 children, with cervical descent)

One of the practices performed by a TBA during delivering a baby was that the TBA rubbed the vaginal canal of the mother and the presenting part of the baby with oil to enhance smooth delivery. Another practice was that the TBA cut the perineum of the mother with a sharp instrument (like performing an episiotomy), again to enhance smooth delivery. However, no stitching was performed. Mothers were also happy with the situation, because they would not encounter difficulties in future labours.

Reasons for not seeking treatment for the disability conditions included feeling ashamed to go to health staff or to any other person and considering the condition was not so serious. Generally they reported the disability conditions to a very close person, and the feedback they received was that such conditions were natural for women after deliveries. Such feedbacks were meant for urethrocoeles, cystocoeles and rectocoeles. However, cervical descents and perineal tears were considered conditions

needing repairs. Among these mothers, unaffordability was the main reason for not seeking treatment for the disability conditions they were suffering.

“I was told that it would cost about Kyat 30,000 if I take surgical treatment at the hospital ... I cannot afford for that”

(A 26-year old mother of 1 child delivered with a TBA, and having a cervical descent)

Cases with vesico-vaginal fistulae

Case 1

Ma Sint (not her real name) is a 38-year old mother from a *let-loke-let-sar* (manual labourer) family. She got married when she was 19 years old and was 5 times pregnant. She has 4 children alive, all delivered by a TBA. Her fifth child was born dead. During delivery of the last child a TBA was called first, but was referred to the hospital for prolonged labour. According to Ma Sint, she started suffering from urine leakage 14 days after her last dead baby was delivered at the hospital. On further investigation, it was found out that she had a Caesarean hysterectomy for ruptured uterus 7 months prior to the interview date. She has a small cystocele and a second degree perineal tear with vesico-vaginal fistula on the anterior vaginal wall.

She is still staying with her husband of first marriage. She considers her health state good; has no serious social problems; and is optimistic about her future.

“I do not have any problems with my husband. Some of my friends make joke on me saying that I am having urinary smell. This situation affects my income because I cannot work as before to earn our family’s living. I went back to the hospital where I got this injury ... they told me to go to Yangon for tretment ... my family cannot afford for the expenses”

Case 2

Ma Myint (not her real name) is a 35-year old mother from a relatively well-to-do family. She got married when she was 25 years old and was 6 times pregnant. She had abortion with her fifth pregnancy. She has 5 children alive out of which 4 were delivered by a traditional birth attendant at home. During delivery of the last child she was referred to the hospital for prolonged labour. According to Ma Myint, she started suffering from urine leakage 10 days after her last baby was delivered at the hospital by Caeserean section. She has a moderate cystocele, rectocele and second degree perineal tear with vesico-vaginal fistula on the anterior vaginal wall.

She is still staying with her husband of first marriage. She considers her health state good; has no serious social problems; and is optimistic about her future.

Note: Ma Myint accepted the referral made by the survey team to Yangon General Hospital and underwent surgical repair.

DISCUSSION AND CONCLUSIONS

There was formerly no data available on obstetrics-related disabilities for rural women in Myanmar. Our findings indicated that 83% were having cystoceles, 22% with urethroceles, 22% with cervical descent, 32% with rectoceles, 32% with perineal tears and 14% with enteroceles. The higher prevalence of four disability conditions (cystocele, urethrocele, cervical descent, and rectocele) among 30-44 age group has more biological meaning than social meaning, as these conditions can be expected to become more prevalent in higher age and higher parity groups. Vaginal delivery is the commonest cause of prolapse as it traumatizes the pelvic floor muscles, ligaments and fascia and causes pudental nerve damage, resulting also in urinary and faecal incontinence in addition to prolapse [7].

Only 13% of women with disabilities sought care. Many women suffering from prolapse do not seek medical advice even in developed countries; this makes difficult to define the incidence of prolapse in the female population. Clinical examination does not necessarily correlate with symptoms and prolapse is frequently an asymptomatic incidental finding [8]. About 50% of parous women have prolapse, which is symptomatic in 20%[7].

About 75% of the women admitted that their disabilities were having some impacts on certain aspects of their lives. Our study findings indicate that majority of rural women, irrespective of their age, education and occupation, tolerated their disability conditions.

Fifty five percent of the birth deliveries made by rural women in our study were attended by TBAs and other untrained accouchers. This finding highlights the important roles TBAs are still playing in rural areas.

In our study, we discovered only two women with vesico-vaginal fistula. In developing countries over 90% of fistulae are of obstetric origin and are due to pressure necrosis from prolonged obstructed labour [9]. The fact that the patients were delivered by Caesarean section does not automatically exclude pressure necrosis as the damage may have occurred as a result of prolonged before the Caesarean section. Obstetric fistulae are more common in young primigravid women but neither age nor gravidity is exclusive [10].

Our study findings call for attention to be paid to various kinds of physical disabilities that Myanmar rural women are suffering following births; and also for consideration to impart TBAs with safe and clean delivery skills. The message that repeated stretching and damage to the uterine and vaginal muscles lead to genital prolapses should be informed to TBAs, auxiliary midwives and midwives through safe motherhood training programmes, and through these birth

attendants to pregnant mothers and community. Appropriate strategies that have reduced maternal mortality and morbidity should be tried. Studies have indicated that maternal mortality can be lowered by introducing trained TBAs and ensuring their regular in-service training[11]; and that the problems associated with obstructed labour can be reduced by using maternity waiting homes where “at risk” women can reside during the later weeks of pregnancy in close proximity to expert assistance[12,13].

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**Detection of cyanogenic glycoside in *Acalypha indica* Linn.
(Kyaung-hsay-pin)**

*Khin Tar Yar Myint, *Mu Mu Sein Myint, **Win Myint, *May Aye Than,
*Aye Than, *Win Win Maw & *Thandar Myint

*Pharmacology Research Division
Department of Medical Research (LM)
**Department of Traditional Medicine (Yangon)

Medicinal plants and herbal drugs are being increasingly utilized for health purpose throughout the world. Also in Myanmar, many of the traditional remedies are accepted and used by a large segment of the population. Generally, people believe that herbal medicines are effective and have no toxic effect. Kyaung-yo-thay (or) Kyaung-hsay-pin is one of the well-known Myanmar medicinal plants used for bronchodilating and mucolytic activities. Its botanical name has been identified to be *Acalypha indica* L. This plant was reported in some literatures to contain cyanogenic glycoside. In order to prove the presence of toxic cyanogenic glycoside in Kyaung-hsay-pin, a highly sensitive color reaction test for cyanogenic glycoside was carried out on the various fresh and dried parts of the plant specimens. It was found that a significantly higher amount of cyanogenic glycoside was present in fresh specimens of plants as compared to the smaller amount present in dried specimens. Expressed juice of leaves was also tested for its acute toxicity administering orally to mice and rats. The results were compiled and discussed.

INTRODUCTION

A medicinal plant known as Kyaung-yo-thay (or) Kyaung-hsay-pin has been known to have possibly toxic effect when the fresh plant expressed juice was taken orally for medicinal purpose [1]. Studies [2, 3] have identified Kyaung-hsay-pin to be *Acalypha indica* Linn., and that the presence of cyanogenic glycoside in this plant has been mentioned as the possible cause of toxic effect. *Acalypha indica* Linn. (Euphorbiaceae) grows widely throughout the whole Myanmar and has been widely used for medicinal purposes [4]. Roots has been used as cathartics, and leaves have been used for scabies, snake bite and, as mild laxative in constipation [4]. The whole plant is reputed to have activities like anthelmintic, expectorant, emetic, anodyne and hypnotic. Fresh or dry plant is claimed to be effective for gastrointestinal disease and has been used as a substitute for

ipecacuanha [5]. Some people used expressed juice of fresh leaves and decoction of dry leaves for bronchodilating activity. Since the plant is well known for its usefulness in spite of its likely toxicity, there is a need to explore the true toxic effect of its constituents so that its therapeutic status as a medicine will become well established.

Objectives

- To detect cyanogenic glycoside in fresh and dried specimen of Kyaung-hsay-pin
- To test its toxic effect on mice and rats.

Myanmar Name : Kyaung-hsay-pin (or)
Kyaung-yo-thay

Botanical Name : *Acalypha indica* Linn.

Family : Euphorbiaceae

Species : Indica

Genus : *Acalypha*

English Name : Indica *Acalypha*



Fig. 1. *Acalypha indica* Linn.

MATERIALS AND METHODS

Plant specimen was procured from the local traditional medicine shops. Both the freshly collected samples and the air-dried samples were subjected to chemical characterization and toxicity on mice and rats.

1. Chemical detection

Detection of cyanogenic glycoside from tested samples was carried out referring to the methods stated as follows.

Method I [Guignard test]

This test [6] was carried out by putting 2-5 g of plant material in a test tube and sufficient amount of distilled water was added to moisten the sample. Filter paper soaked in sodium picrate was inserted above the plant sample, and the test tube stoppered and warmed at 35°C for 3 hours. The presence of cyanide was noted by a change in colour of the yellow picrate test paper to brown.

Method II

The extracts of fresh and dried specimens were tested by Thin Layer Chromatography using precoated silica gel glass plate. TLC system for cyanogenic glycoside was n-butanol:ethanol:water(40:11:10) and detected on UV radiation at 254 nm [7].

2. Acute toxicity test

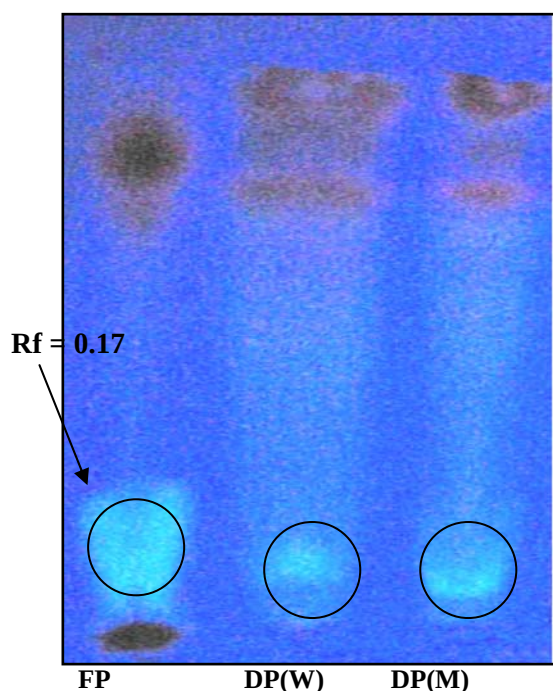
A total of 50 albino mice of ddy strain weighing 30~35 gm were issued from Laboratory Animal Services Division. They were fasted overnight but water was allowed *ad libitum*. Animals were divided into 5 groups of 10 each. Four drug dose levels (40, 50, 60 and 80 ml/Kg) were administered orally. One group received only distilled water, which was served as negative control group. The mice were then continuously observed for their mortality and behavioural responses for 4 hours and there after once daily until the 14th day. The death rates were observed and noted. LD50 was calculated by the method of Litchfield and Wilcoxon, 1948 [8].

RESULTS

Cyanogenic glycoside detection by Method 1 indicated the presence of cyanide compound by the development of a brown color. Fresh leaves and stems showed a more intense deep-brown color than fresh root specimens. Similarly, dried leaves and stems showed a deeper color development than roots.

It was observed that, weight by weight, the content of cyanogenic glycoside was higher in the fresh specimen than the dried specimen as noted from the deep brown color developed in the tests. Similarly, fresh and dried specimen of the whole plant also indicated that cyanogenic glycoside content was higher in the fresh specimen than in the dried specimen.

In the second method (Method II), expressed juice and methanol extract of dried plant were tested by TLC method. Rf value of fluorescence compound indicated cyanogenic glycoside in expressed juice was 0.17. Thin layer chromatogram is shown in Fig. 2.

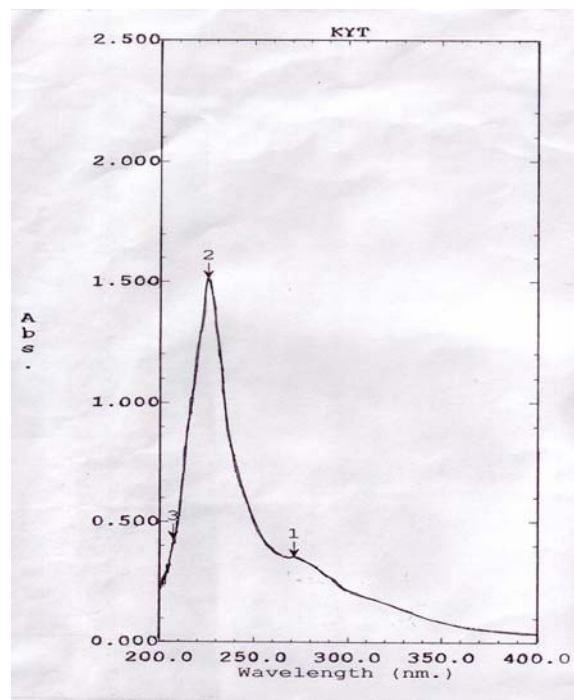


FP = Fresh plant juice
DP(W) = Watery extract of dry plant
DP(M) = Methanol extract of dry plant

Fig. 2. Thin Layer Chromatogram of Kyaung-hsay-pin extracts

The compound shown in Rf = 0.17 was the scraped off from the TLC plate and reconstituted in methanol. This was further scanned in UV radiation within the wavelength range of 200-400nm. Determination of wave length by UV spectrophotometer (DU 650) showed a UV spectrum at 223nm and a shoulder at 258nm (Fig. 3).

In acute toxicity test, behavioural changes observed after the oral administration of the fresh juice included vomiting, loss of locomotor activities and eventual death within 24 hrs. No significant changes were seen in the organs when observed by naked eye. The estimated LD50 value for the fresh juice was found to be 79 (56.8–109.8) ml/kg in mice when administered orally (Table 1).



Peak No	Pick wavelength(nm)	Abs.
1	258	0.355
2	223	1.5128
3	206	0.4268

Fig. 3. UV spectrum of cyanogenic glycoside

Table 1. Results of acute toxicity tests of fresh juice of *Acalypha indica* Linn. on albino mice and rats

Times to No.	human dose	Dosage ml/kg (1ml= 0.07g)	Mice		Rat	
			No. of death observed	% death	No. of death observed	% death
1	200 times	80 ml/kg	6/10	60%	not done	Not done
2	150 times	60 ml/kg	4/10	40%	not done	Not done
3	125 times	50 ml/kg	2/10	20%	4/5	80%
4	100 times	40 ml/kg	1/10	10%	not done	Not done

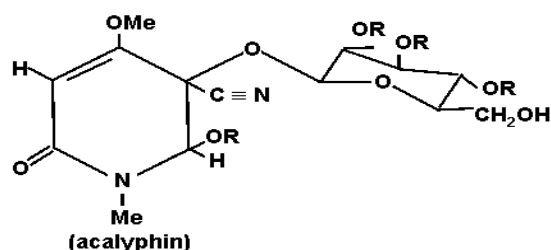
Five rats were administered with 50 ml/kg of fresh juice orally and were observed for 2 weeks; 4 rats died within 24 hours.

Detection of cyanide was also carried out in blood collected from cardiac chamber of the rat which died after administration of tested expressed juice. Blood specimen was acidified with sulphuric acid and release of hydrogen cyanide gas was detected by the

same picric paper method [9]. Yellow picric paper changing to brown, indicated the presence of cyanide in the test blood sample.

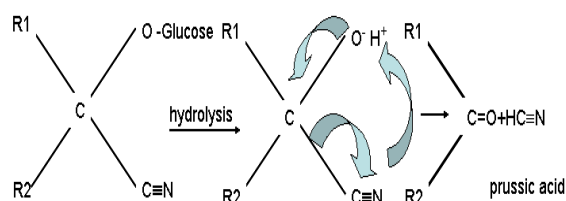
DISCUSSION

The presence of cyanogenic glycoside compounds in Kyaung-hsay-pin was preliminary detected by colour reaction. Trace amounts of fluorescence compound was collected and determined for its UV spectrum. Although the authentic sample was not available, acalyphin of the Kyaung-hsay-pin was identified by UV spectral data reported in the literature [10]. A peak at 223 nm and shoulder at 258nm of the isolated compound was observed to be very close with those of acalyphin at 223nm and 255nm. Therefore the cyanogenic glycoside of Kyaung-hsay-pin was identified as acalyphin. Its structure is shown below.



Living plant tissues of *Acalypha indica* Linn. contain cyanogenic glycoside and (b-D glycoside) in separate cells. When plant tissues are damaged, by chopping or grounding, enzymes ultimately come in contact with the cyanogenic glycoside that is released and result in the formation of prussic acid (HCN) [11].

The production of HCN from cyanogenic glycoside can be explained by the following mechanism.



$\text{C} \equiv \text{N}$ is a weak conjugated base, that is very stable. SP character donation power of the electron pair on carbon atom became very low. The combination of H^+ and: $\text{C} \equiv \text{N}$ anion leads to $\text{HC} \equiv \text{N}$. Accordingly, reaction with cyanogenic glycoside compound present in Kyaung-hsay-pin will certainly occur. The present study proved that Kyaung-hsay-pin, used as folklore medicine in Myanmar contain toxic cyanogenic glycoside compound. It had caused death in tested animals at the dose of 125 times of human dose (50 ml/kg) in 20% of mice and 80% of rats. It was assumed to be the cause of death since a significant amount of cyanide was detected in the rat blood from the cardiac chamber.

Once cyanide gets into the blood, it is readily distributed throughout the body. It is extremely toxic to all animals. In cells, cyanide reacts with cytochrome oxidase (an enzyme involved oxygen) to form a stable, inactive complex. As a result, the cyanide ion inhibits the release of oxygen from the hemoglobin of blood to individual cells. Without oxygen, cellular respiration ceases and cells die rapidly due to hypoxia [11]. Moreover, *Acalypha indica* Linn. was found to induce haemolysis in glucose-6-phosphate dehydrogenase deficiency [12].

CONCLUSION

The present study had proven that Kyaung-hsay-pin contains cyanogenic glycoside which can produce toxic cyanide compound. Expressed juice of fresh leaves and stem can cause death of mice at the dose of (80 ml/kg) but the extract of dried specimens at the same dose did not cause death in animals, since the cyanogenic glycoside content is more in fresh plants than in dried specimens. Consequently fresh specimen of Kyaung-hsay-pin is more toxic than dried specimens according to the chemical and toxicity data.

Finally, it was recommended that cyanogenic glycoside and related toxic effect should be seriously taken into consideration

whenever Kyaung-hsay-pin is used as folklore medicine or for any other edible purposes.

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Prospective study of seroepidemiology: persistent seropositives developed leprosy

Khin Nwe Oo, Nwe Nwe Yin & Tin Tin Han

Immunology Research Division
Department of Medical Research (Lower Myanmar)

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

INTRODUCTION

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

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

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

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

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

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

MATERIALS AND METHODS

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

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

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

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

RESULTS

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

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

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

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

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

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

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

N¹ = number of seropositives

N² = number of tested subjects

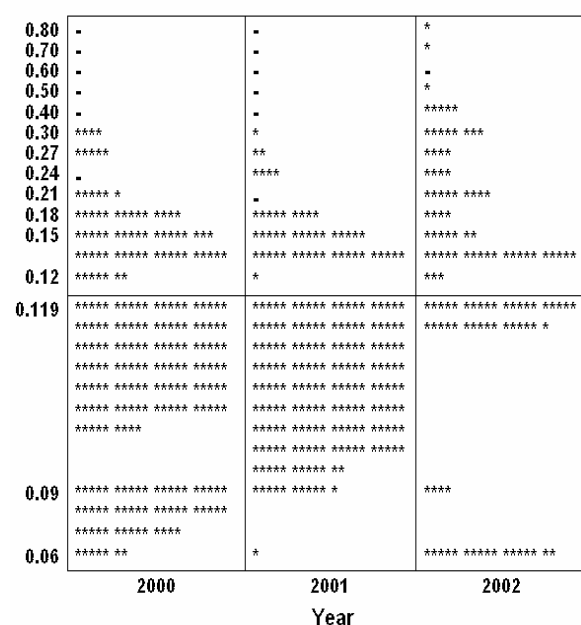


Fig. 1. Scattergram of the OD values of anti IgM, NTP-BSA antibodies titers of villagers' sera of Kyanbokone village

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

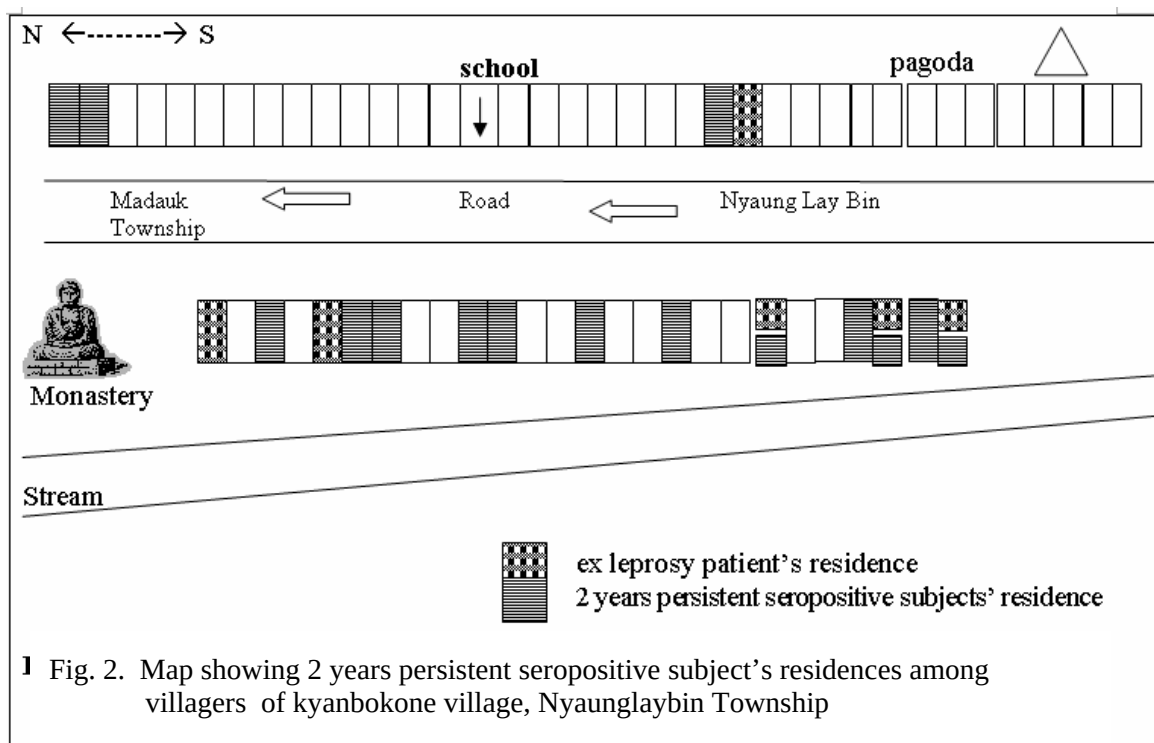
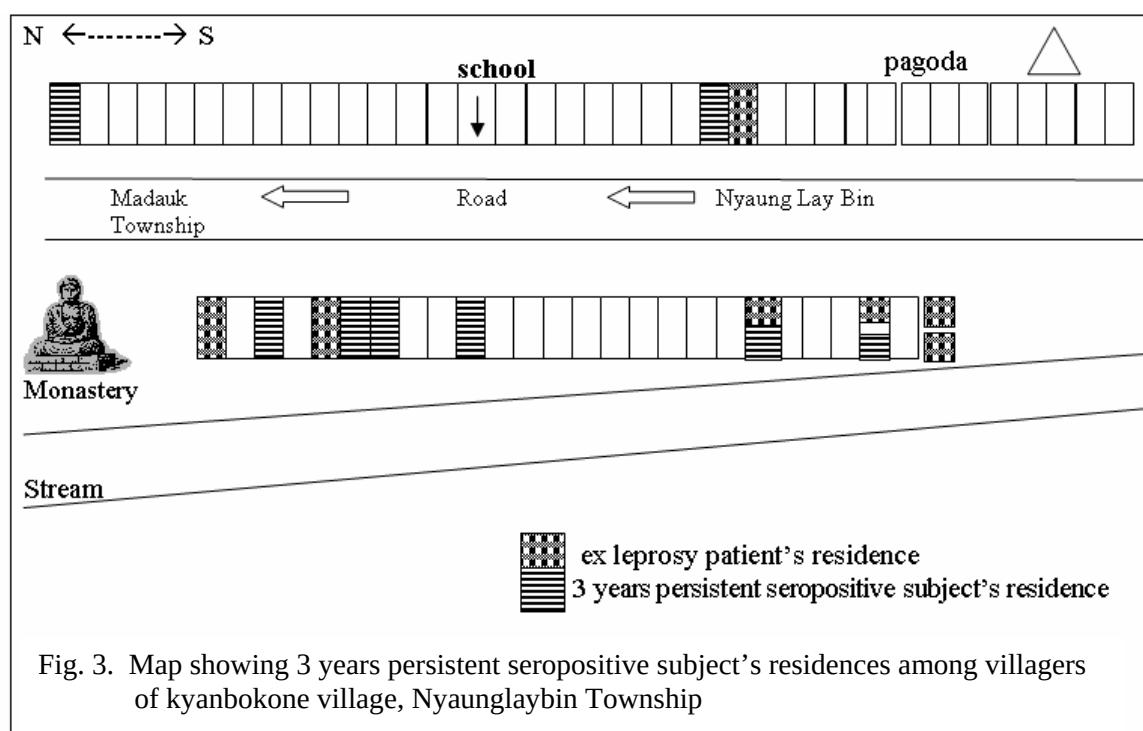
As shown in Figs. 2 and 3 most of the persistent seropositives were household and neighbor contacts of the old patients. There were 17 and 8 subjects of 2 and 3 years persistent seropositives respectively in our

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

DISCUSSION

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

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



ACKNOWLEDGEMENT

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**Food and beverage consumption behaviour in Mandalay and Patheingyi:
A quantitative study**

**Phyu Phyu Aung, *Theingi Thwin, *Thandar Shwe, *Thidar Khine, *Aye Myint Oo,
*Khin Thida Wai & **Than Tun Sein*

**Nutrition Research Division, DMR (LM)*

***Department of Medical Research (Lower Myanmar)*

The aim of the study is to find food and beverage consumption behaviour and the factors effecting on the behavior of urban populace of Mandalay and Patheingyi and to compare among different occupational groups. The study was carried out from September 1998 to November 1999. Using the findings of previous qualitative study, a structured questionnaire was constructed and meal planners/housewives of randomly selected household of the said cities were interviewed, face-to-face by the trained interviewers. A total of 429 and 409 subjects were interviewed in Mandalay and Patheingyi, respectively. Most of them took two meals a day and either meat or fish was included almost daily. Fried rice and Moh-hin-ga were the two kinds of food most commonly taken for the breakfast, in both cities. Mid-day snacks and coffee/tea were taken more in persons of Mandalay. Milo/Cocoa/Horlicks etc. were mostly taken during illness. More than 50% of the respondents had green tea after their meals. Imported packed snack foods were taken by very few. Habit of taking soft drinks was found to be more common in Mandalay than in Patheingyi. (Although some like "Yet Saar", they only took sometimes). Monosodium glutamate was found to be taken by almost all households and average amount they used for cooking at home was 1.64g/day with a range of 0.1 to 10.4g/day. Palm oil was used as cooking oil mostly in families of unskilled labourers while ground-nut oil was mostly used by other occupational groups. Very few bought ready-made foods and went to supermarket to buy food. None of them took a kind of food for health reasons although there were many who avoided taking some foods for their health. Avoidance of food was found to be widespread for various reasons. Breast-feeding was practiced in more than 80% of respondents in both cities. Working more was the main mechanism to cope with rise in price of commodities.

INTRODUCTION

Food consumption behaviour is difficult to describe and predict. The net effect on food intake depends on a wide range of factors. Apart from income, demographic characteristics such as urbanization, gender, ethnicity, education, employment status, food availability, and other factors such as culture and beliefs, mass media advertisements, family resource management, residence, market price, and food policy can also be important because of the way they relate to consumption or concurrently co-effect consumption with income [1,2].

Essentially, the proportion of income expended on food is reduced as income decreases. In contrast, when income increases more money is spent on more elaborate packaging and processing or on high quality of specific foods rather than on larger quantities of food or shifts in types of food, changes in income have little effect on dietary structure [3, 4].

Patheingyi and Mandalay are the capital cities of Ayeyawady and Mandalay Divisions of Myanmar; much urbanized. There are a lot of food advertisements from the mass media like television which can effect the kinds of food

intake. We are under the impression that concomitant with the change in socio-economic conditions there is a change in the food and beverage consumption behaviour/habits of the city population as a whole which might have either positive or negative impact on the nutritional status of the population.

The present study, thus, aims to determine food and beverage consumption behaviour of different occupational categories during the socio-economic transition period which can serve as a basis for the food planners and food policy makers in food and nutrition planning of Myanmar. The information provided can be used to develop intervention strategies for improvement of malnutrition problems in Myanmar.

Research approach and study design

The study was community-based, descriptive, and exploratory in nature. A household survey was conducted using a structured questionnaire. Face-to-face interviews were conducted by trained data collectors to elicit information on the basic socio-demographic characteristics, food and beverage consumption behaviour, and factors influencing the certain behaviour. Questionnaire was constructed based on the findings from the focus group discussions conducted before.

1. Study area and study population

Two urbanized cities of Myanmar namely; Mandalay, and Pathein were the sites of study. Both are the capitol cities of the respective divisions of Myanmar. They had been chosen as the food behaviour changes would be most likely to occur in areas which are more urbanized and where impact of socio-economic changes on food behaviour would be more.

2. Method of sampling

Multistage sampling method was used. For the first stage, two states and divisions were randomly selected among the 16 from Myanmar. Capitol cities of the chosen states and divisions were selected for reasons of

being capitol and urbanized. From the selected cities, ten wards were randomly selected from each city. Then, five percent of the total households in the respective wards were again randomly selected and the meal planners/housewives of the selected households were interviewed with the structured questionnaire.

Data management/analysis

Prior to data entry, range and consistency checks were done and errors were corrected with regards to the survey questionnaire. Data entry was then done by using Epi Info Version 6. Findings were analyzed by employing frequency distribution tables and cross-tabulations on selected variables.

Findings of the quantitative study in Pathein and Mandalay

1. General characteristics of respondents of Pathein and Mandalay

The socio-demographic data of the respondents are summarized in Table 1. A total of 429 and 409 households were sampled from 9 wards of Pathein and 6 wards of Maha-Aung-Myay Township of Mandalay. Maha-Aung-Myay had been chosen as families of different occupational groups reside. Meal planners from the randomly selected households were interviewed. Age ranged between 15 and 82 years were included; most of them were between 21 to 60 years (87.3% & 92.7% respectively in Pathein and Mandalay).

For occupation, private business entrepreneurs were rather scarce in Pathein and most of the respondents were unskilled labourers and government employees. In Mandalay, 51% came from families of private business entrepreneurs as business sector is more sprouted in Mandalay.

Majority of the respondents (83.6%) of Mandalay were middle income earners whereas in Pathein only 60.6% were middle income earners and they had more of low income earners of 38.7% (ct 7.5% in Mandalay).

Table 1. Characteristics of study population

No.	Category	Pathein No. (%)	Mandalay No. (%)
1	<u>Age</u>		
	15-20 yr	6 (1.4)	0
	21-40 yr	160 (37.3)	133 (32.5)
	41-60 yr	218 (50.8)	246 (60.2)
	61-82 yr	45 (10.5)	30 (7.3)
2	<u>Main occupation of family</u>		
	Unskilled labourers	169 (39.4)	128 (31.3)
	Government employee	105 (24.5)	43 (10.5)
	Private business	76 (17.7)	208 (50.9)
	Private company Employee	47 (11.0)	1 (0.2)
	Farm labourers	5 (1.2)	6 (1.5)
	Farm owners	4 (0.9)	0
	Others	23 (5.4)	23 (5.6)
3	<u>Income</u>		
	*Low income	166 (38.7)	31 (7.5)
	Middle income	260 (60.6)	342 (83.6)
	High income	3 (0.7)	36 (8.8)
4	<u>Religion</u>		
	Buddhist	392 (91.4)	402 (98.3)
	Christian	9 (2.1)	4 (0.9)
	Muslim	27 (6.3)	-
	Others	1 (0.2)	3 (0.7)
5	<u>Ethnic</u>		
	Bamar	375 (87.4)	384 (93.9)
	Other nationalities	54 (12.6)	25 (6.1)
6	<u>Marital status</u>		
	Married	320 (81.8)	314 (76.8)
	Widow	62 (14.5)	44 (10.8)
	Single	32 (7.3)	28 (6.8)
	Divorced	16 (3.7)	23 (5.6)
7	<u>Educational attainment</u>		
	Illiterate	45(10.5)	49 (12.0)
	Able to read/write	41(9.6)	60(14.7)
	Primary level	153(35.7)	125(30.6)
	Secondary level	99(23.1)	76(18.6)
	High school level	63(14.7)	62(15.2)
	University level	28(6.5)	37(9.0)

* 500-5000 K = Low income
 5000-30000 K = Middle income
 Above 30000 K = High income

More than 90% of the study population were buddhists, followed by muslims (6.4%) in Pathein and christians (0.9%) in Mandalay. Marital status in two cities were not much different although divorcees were found to be present more in Mandalay.

Table 2. Meal frequency and types of food taken in daily main meals

Types of food	Pathein No. (%)	Mandalay No. (%)
<u>Meal frequency/day</u>		
1	1 (0.2)	4 (1.0)
2	360 (83.9)	237 (57.9)
3	62 (14.5)	159 (38.9)
4	6 (1.4)	8 (2.0)
<u>Types of food taken</u>		
<u>Main meals</u>		
Meat/fish & vegetables	129 (30.1)	218 (53.3)
Meat/fish & vegetables & fishpaste	128 (29.8)	53 (13.0)
Vegetables & fishpaste	98 (22.8)	44 (10.8)
Meat/fish & fishpaste	46 (10.7)	11 (2.7)
Others	28 (6.5)	83 (20.3)
<u>Breakfast</u>	196 (45.7)	183 (44.7)
Fried rice	157 (36.6)	37 (9.0)
Moh-hin-ga	14 (3.3)	5 (1.2)
Not usually take breakfast	35 (8.2)	170 (41.6)
Coffee/tea with bread	25 (5.8)	14 (3.4)
Food made of rice/wheat	2 (0.4)	2 (0.4)
Others		
<u>Snacks</u>		
Not usually take	175 (40.8)	107 (26.2)
Fried rice	86 (20.0)	35 (8.6)
Food made of rice/wheat	77 (17.9)	60 (14.7)
Coffee/tea	45 (10.5)	150 (36.7)
Moh-hin-ga	21 (4.9)	16 (3.9)
Fruits	20 (4.7)	28 (6.8)
Ah-thoke and others	5 (1.2)	13 (3.2)

2. Meal frequency and type of foods taken in main meals, for breakfast and snacks

Table 2 shows the meal frequency and types of food taken by the respondents. In Mandalay, more than 50% took 2 main meals a day, while nearly 40% took 3 meals. Four (1.0%) had only one main meal a day, while 2% had four meals a day. In Pathein also the majority took 2 or 3 main meals. In Mandalay, more than 50% of the study population took meat/fish and vegetables in their daily meals, while in Pathein, it was only 30% who consumed meat/fish and vegetables everyday. Almost all took breakfast (except 3.3% in Pathein and 1.2% in Mandalay). In Mandalay, commonly taken food for breakfast were fried rice and coffee/tea with bread, 44.7% and 41.6%

respectively. Less commonly taken foods were moh-hin-ga, and other snacks made from cereals. Most of the persons used to have more than one type of food for breakfast. In Pathein, fried rice and moh-hin-ga were the most popular food. Snacks were usually taken by children and young adults. Since our study population were meal planners who were more in within 40 and 60 years of age, about 40% in Pathein and about 25% in Mandalay said that they did not take snacks usually. The rest took snacks regularly. Coffee/tea and Myanmar traditional snacks made from rice/wheat were the most common eatables as midday/bedtime snacks in both cities. Moh-hin-ga, ah-thoke, and fruits like banana, groundnut, sweet potato were the other less commonly taken food.

3. Habit of taking coffee/tea

The habit of taking coffee/tea was found in 80.7% of the study population of Mandalay and 62% in Pathein. Among them only 56.5% and 14.9% in Mandalay and Pathein took daily respectively. From these coffee/tea drinkers about 25% preferred to have coffee/teamix. Milo, cocoa, and ovaltine intakes were found in less than 20% of the subjects in both areas. For the soft drinks, around 50% in Pathein and 40% in Mandalay had the habit of consumption. Only about a quarter of the study population (24% in Pathein and 28.1% in Mandalay) had the habit of taking imported snack food.

Most of them took the snack foods frequently. Most commonly taken packed snack foods were Yum Yum, other types of dried noodles and Jumbo. Less percent of subjects in Pathein had the habit of taking cholesterol rich foods. Even those who had habit of it, only few consumed these foods regularly. Less than half (34%) of the study population in Mandalay and 50% in Pathein had the habit of taking ready-to-eat food which were mostly dried noodles packages, especially during illness.

4. Oil and monosodium glutamate consumption

About 87% of the families of Mandalay used ground nut oil as their cooking oil, while only

Table 3. Habit of taking drinks, snack foods, cholesterol rich foods and ready-to-eat/fast food

Habit	Pathein No. (%)	Mandalay No. (%)
<i>Coffee/Tea</i>		
Yes	266 (62.0)	330 (80.7)
Daily	64 (14.9)	231 (56.5)
Frequently	44 (10.3)	86 (21.1)
Sometimes/rarely	158 (36.8)	13 (3.2)
No	163 (38.0)	79 (19.3)
<i>Milo/Cocoa/Ovaltine</i>		
Daily	7 (1.6)	10 (2.4)
Frequently	13 (3.0)	29 (7.1)
Sometimes/rarely	50 (11.6)	16 (3.9)
<i>Soft drinks</i>		
Daily	10 (2.3)	5 (1.2)
Frequently	24 (5.6)	124 (30.3)
Sometimes/rarely	191 (44.5)	38 (9.3)
<i>Imported packed food</i>		
Yes	103 (24.0)	115 (28.1)
Daily	9 (2.1)	7 (1.7)
Frequently	16 (3.6)	83 (16.1)
Sometimes/rarely	78 (18.1)	25 (6.1)
No	326 (76.0)	294 (71.9)
<i>Cholesterol rich foods</i>		
Yes	73 (17.0)	122 (29.8)
Always	3 (0.7)	8 (2.0)
Frequently	5 (1.2)	20 (4.9)
Sometimes/rarely	65 (15.2)	94 (23.0)
No	356 (83.0)	287 (70.2)
<i>Ready-to-eat/fast food</i>		
Yes	73 (17.0)	122 (29.8)
Always	3 (0.7)	8 (2.0)
Frequently	5 (1.2)	20 (4.9)
Sometimes/rarely	65 (15.2)	94 (23.0)
No	356 (83.0)	287 (70.2)

31.5% in Pathein used it. The majority of the families in Pathein used palm oil. Sesame, and other types of oil were used by the rest. Mean value of oil intake was found to be 32.9g per head per day in Mandalay and 20.3 g per head per day in were Pathein. On average 1.3g, range (0.1-8.0g) of monosodium glutamate per head per day in Mandalay and average of 1.34g, range (0.1-10.0g) per head per day in Pathein were used in cooking. That was much higher than in USA where average consumption was only 0.4 g/head/day but less than in South Korea

where it was more than 3 g/head/day. This calculated amount was only from intake of food made at home. The actual intake would be higher if one takes into account food having outside. The intake of mono-sodium glutamate was comparable between the two cities.

5. Factors considering in buying food for main meals

Table 4. Factors considering in buying foods for main meals

Factors considered	Patheingyi No. (%)	Mandalay No. (%)
Price	219 (51.0)	80 (19.7)
Taste and preference	136 (31.7)	225 (55.0)
Advertisement	1 (0.2)	1 (0.2)
Nutrition	3 (0.7)	1 (0.2)
Others	7 (1.6)	9 (2.3)
More than one factor	67 (15.6)	86 (21.0)

Most of them stated that they considered taste and preference first in buying food for their main meals in Mandalay, while 19.7% answered that price was the main factor they considered.

In Patheingyi, price was the main factor. Some considered more than one factor. The other few reasons were being nutritious, healthiness, advertisement and others.

6. Breastfeeding and weaning practice

Breastfeeding was practiced in 84.5% in Patheingyi and 75.3% of the subjects studied in Mandalay. Those who did not breastfeed use varieties of milk powder or condensed milk. Mean duration of giving breast feeding was 18.5 months with a range of 1 to 84 months in Mandalay and 16.1 months with a range of 2 to 60 months in Patheingyi. For the working mothers, after the puerperium, mixed bottle and breastfeeding was given. They used different kinds of fortified cow's milk especially those which were less expensive in the particular time. Complementary feeding was started around 3 to 4 months (mean±4.5 months) in Mandalay and 3 to 6 months in Patheingyi. Boiled rice with beans/potato/banana, jaggery, chicken egg, biscuit and locally available instant weaning food were given except very few mothers who stated that they

gave "Nestum" for complementary feeding. Most of the respondents had the knowledge that by 4 months babies should be given complementary feeding. But most of the food had low protein content.

7. Food avoidance

Almost two third of the study population in both cities (68.1% in Patheingyi and 66.0% in Mandalay) had the habit of avoiding food during illness, pregnancy, lactation and even in normal conditions. Most of them avoided beef (50.1%) for religious reason. Pork was also found to be avoided in nearly 50% of the respondents in both cities, the reasons were belief in "Nats, being Muslims and not like the taste. Other food that were avoided were some kind of fish like eel, brinjal, banana eggs etc during pregnancy and lactation.

DISCUSSION

Food consumption behaviour and proper food intake is multifactorial in nature including, as found in this study, occupation/income, education, health status, culture and beliefs, food prices, media advertisement etc. To improve the food behaviour of a community, food and nutrition planners should look holistically and pay attention to every possible angle that would lead to improper food consumption behaviour and intake [3].

Patheingyi, although a capital city of Ayeyawady Division is not very much urbanized as Yangon/Mandalay etc, and thus supermarkets, fast food centres, and burger shops alike are not available. People took Myanmar traditional food and habit of taking snacks and soft drinks were not common. Respondents, being the meal planners/housewives might also be the explanation for the above.

Majority of the respondents (83.6%) of Mandalay were middle income earners whereas in Patheingyi only 60.6% were middle income earners and they had more of low income earners of 38.7% (vs 7.5% in Mandalay). This can also be explained by the fact that business is more fruitful in Mandalay than in Patheingyi which is also apparent by the

finding of different percent of income levels in two cities.

The present study provided the following essential information :

- Food avoidance for various reasons is common
- Snacks are not widely taken among the meal planners/ housewives
- Drinks like milo, cocoa, ovaltine etc are not regularly consumed except during the illness
- Respondents preferred buying foods in the ordinary markets
- Families of unskilled laborers are the most vulnerable group to suffer from undernutrition
- Breastfeeding is a common practice among the Myanmar women but cereal-based foods are mainly used as the complementary food for babies.
- Myanmar traditional foods are mostly consumed in Pathein, while coffee/tea are commonly taken in Mandalay among the meal planners/housewives

In the past, women were not very enthusiastic to attain high education level and that might be the reason why there were very few respondents with university level education in both cities.

The findings in both cities highlight the fact that Myanmar usually take 2/3 main meals a day. Type of food taken in two cities were more or less similar, although those who took vegetables and fishpaste only were more in Pathein. This might also reflect their income pattern. Main meals in Pathein usually included fish paste as it is the traditional food of the delta area. Compared to the unskilled labourers, other occupational groups took meat/fish everyday more. Only about 15% in Pathein were found to be daily drinkers. That may be due to more business families in Mandalay and from the past experience coffee/tea were taken more often in those who did business.

In our country, milo/ cocoa/ ovaltine drinks were only taken during the illness. Thus it is

not surprising that most of the respondents did not take them regularly. Only 16.3% and 13.4% in Pathein and Mandalay respectively used to take milo/cocoa/ovaltine etc. Among them only 1.6% in Pathein and 2.0% (10 persons) in Mandalay had the regular habit of taking them.

For the soft drinks, about 50% in Pathein and 40% in Mandalay took them, and usually frequently in Mandalay than in Pathein which may be due to the hot weather. Soft drinks had become popular lately in Myanmar because of affordable prices as many local brands were produced. Snacks are mostly taken by children and young adults thus finding of low intake of snacks in our study might be due to age group selected. People in Myanmar usually did not frequently take cholesterol rich food like butter, cream, yogurt, cheese which were taken by 29.8% of the study population of Mandalay and 17% in Pathein. Better income of the Mandalay respondents might be the explanation for this different finding. By occupation, private business persons and private company employees took most, while none of the farm owners and farm laborers took cholesterol rich food.

People from Mandalay are famous for liking in oil and this explained the higher consumption of oil in Mandalay. Taking more of groundnut oil rather than palm oil in Mandalay also reflects their higher income level as palm oil is cheaper than groundnut oil. Better income among the respondents of Mandalay could explain the thinking of taste and preference in buying foods rather than price in contrast to Pathein where price was the most common factor to be considered in buying foods for main meals.

We, Myanmar consider cows as our "Kyay-zu-shin" (human beings owe a gratitude to cows) as they are mainly used in producing rice, a staple food of Myanmar. Thus we would not like to kill them and eat. Food avoidance was found in all occupation groups, indicating that people still need to be given proper nutritional and health education.

Some reasons for avoiding food were rather deep-rooted and would be difficult to remove the taboos and wrong ideas especially when concerned with religion.

The present study, being the first in Myanmar during the socio-economic transition period, will serve as the baseline for further quantitative and qualitative research studies on food consumption behaviour in wider scope, another studies and periodically, should be carried out to evaluate the impact of developmental transition on food patterns, trends over years, and to map out an agenda of priorities and goals for the turn of the century.

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SHORT REPORT

Assessment of whole cell soluble *Mycobacterium tuberculosis* antigen ELISA (in-house ELISA) test in serodiagnosis of pulmonary tuberculosis

**Khin Nwe Oo, *Nwe Nwe Yin, *Tin Tin Han, **Myat Thida & **Wah Wah Aung*

**Immunology Research Division*

***Bacteriology Research Division*

Department of Medical Research (Lower Myanmar)

A rapid, reliable and inexpensive serological test for diagnosis of tuberculosis would have great applicability as a clinical, epidemiological and public health tool. Therefore, use of whole cell soluble *Mycobacterium tuberculosis* antigen ELISA (in-house test) as a serodiagnostic test for pulmonary tuberculosis was studied.

This study was carried out from February 2002 to January 2003. It was a cross-sectional, laboratory and institutional-based study. Coating antigen was prepared in Tuberculosis Culture Laboratory and Immunology Research Division, Department of Medical Research (Lower Myanmar). It was prepared from *Mycobacterium tuberculosis* cultured on 3% Ogawa media. Whole cell soluble *M. tuberculosis* antigen was prepared using the method of Nicholl's (1975). Phenolized non-viable tubercle bacilli were collected, treated with N sodium hydroxide and heated at 80°C for 30 minutes. After washing and centrifuging, antigen soluble in double distilled water was used as coating antigen in ELISA test.

Sputa and 2 ml of blood samples were obtained from 40 smear positive and 54 smear negative pulmonary tuberculosis cases who attended the Union Tuberculosis Institute, Aung San, during July to September 2002. One hundred and thirty six blood samples were also collected from normal apparently healthy blood donors from National Blood Center (as a control group). Informed consents were taken from

all participants. Sputa were decontaminated and processed into culture at TB laboratory, DMR (LM). Cultures were made onto Ogawa slant under 37°C incubation for 8 weeks.

In-house ELISA test was carried out as follows: Fifty µl of 10 µg/ml concentrated soluble antigens were coated onto duplicate wells, 96-well microtitre plate (Fastec Microplate U. Fujirebio Inc.) and placed at 4°C overnight. Plate was then washed three times with phosphate buffer saline containing 0.05% of Tween 20 (PBST) and 100µl of blocking agent was added to each well and incubated at 37°C for 30 minutes. It was sucked out and 100µl of diluted sera (1:40 dilution) was added to each well and incubated at 37°C for 1 hour and washed with PBST for 3 times. Then 50µl of goat anti-human IgG conjugated horse-radish peroxidase, diluted in 1:1500 with dilution buffer was added to each well and incubated at 37°C for 1 hour. It was washed with PBST for 3 times again. After that, 100µl of substrate solution (orthophenylene-diamine) was added to each well and incubated at 37°C for 15 minutes. Reaction was stopped using concentrated sulphuric acid and readings were taken on an ELISA reader at 492nm. Result was considered positive if OD value was higher than 0.264 (previously calculated cut-off point of control mean + 2 SD).

For smear positive pulmonary tuberculosis group, sputum for culture and in-house

ELISA test were positive in 40/40 and 39/40 samples respectively and therefore sensitivity was 100% and 97.5% respectively. For smear negative pulmonary tuberculosis group, sputum for culture and in-house ELISA test were positive in 29/54 and 38/54 samples respectively and therefore sensitivity was 53.71% and 70.37% respectively. For control group, 114/136 samples were negative in in-house ELISA test and thus specificity of this test is 83.82%.

Therefore, sensitivity of in-house ELISA test on pulmonary tuberculosis in our

community was 97.5% and 70.37% for smear positive and negative pulmonary tuberculosis respectively and specificity was 83.82%. Therefore, this study indicates that in-house test is beneficial for screening of pulmonary tuberculosis (both AFB smear positive and negative cases).

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SHORT REPORT

Bacterial contamination in some local brand milk and milk products bottles

Mar Mar Nyein

Bacteriology Research Division
Department of Medical Research (Lower Myanmar)

World Health Organization has estimated that about 70 percent of diarrhoeal diseases may be due to contaminated food and water and they are responsible for the majority of reported illness annually. However, a great number of illness were unreported. Contaminated water and food increases the risk of enteric infections including cholera, shigellosis and salmonellosis [1-3]. Thus, it is to find out the level of contamination by bacteria in some milk and milk products in bottles with different brands which were prepared locally and sold in the markets.

Milk bottles with different brands prepared locally were collected from marts during March 2000 to September 2001 and transported to the laboratory using cold chain. A total of 78 samples; containing three samples of each product were collected. It comprised of 42 milk bottles (local product with different brands) from 14 places; 9 samples of milk juice from 3 places; 12 samples of yogurt in bottles (local product with different brands) from 4 places and 15 samples of freshly prepared yogurt without ice from 6 places. The controls were the exported brands (Table 1).

Presumptive test by multiple tube technique was done using MacConkey broth purple, with single and double strength, determined the presence of coliforms and faecal coliforms, incubated at 37°C and 44.5°C respectively. Confirmation was done using Brilliant Green Bile medium with their corresponding presumptive positive tube in respective temperatures [4].

Approximately 20 ml of samples were spun and the sediment was inoculated onto Blood, MacConkey, Salmonella-Shigella, Mannitol Salt and Thiosulphate Citrate Bile Sucrose agar for primary isolation. Simultaneously, enrichment media as Selenite F and Alkaline Peptone water were used for secondary inoculation. The suspected colonies were then picked up from respective media and confirmed by biochemical reactions and serotyping [5].

All the tested 42 milk bottles at that time were contaminated with coliforms with faecal coliforms (Table 1). The most probable number per 100ml of milk was higher i.e (>1800MPN/100ml) than yogurt.

Table 1. Bacteriological analysis of pasteurised milk and yogurt with different brands

Type of milk	Tested samples & date	Coliforms	Faecal coliforms	Other bacteria
Milk bottles pasteurized (local packs)	42 (22-5-01 to 7-8-01)	100% (240 - >1800 MPN/100ml)	100% (23 - >1800 MPN/100ml)	EPEC (35.71%) O26K60, O114K90 O148K+, O159K+ Fungi
Milk juice bottles (local)	9 (12-6-01 to 24-7-01)	nil	nil	
Yogurt (local packs)	45 (12-6-01 to 24-7-01)	40% (240 - >1800 MPN/100ml)	40% (17 - 240 MPN/100ml)	nil
Yogurt fresh (local)	15 (19-6-01 to 21-8-01)	2.5% (23 - 43 MPN/100ml)	25%	<i>Lactobacillus</i>
Dutchmill	15-1-01	<2	<2	nil
Viva	15-1-01	<2	<2	nil
Thai	24-1-01	<2	<2	<i>Lactobacillus</i> .

It was also demonstrated that enteropathogenic *Escherichia coli* (EPEC) serotyped O26K60, O114K90, O148K+ and O159K+ were isolated. Although all milk juice samples were neither contaminated with coliforms nor faecal coliforms, fungi was isolated in one sample. The yogurt samples in bottles were contaminated with coliforms and faecal coliforms, however, *Lactobacillus* species was not isolated from the tested yogurt sample bottles. In case of freshly prepared yogurt, the coliform count was significantly lesser than the milk samples ($p < 0.02$); faecal coliforms were not isolated but *Lactobacillus* spp. was isolated (Table 1). The control samples were not contaminated with coliforms and faecal coliforms.

There were reports that outbreak of *Salmonella* serotype Typhimurium infections was associated with drinking unpasteurized milk in USA during 2002-2003 [6]. Foodborne pathogens contamination in milk was reported [7]. Milk is always served as a reservoir for the growth of bacteria. In this study, *Lactobacillus* spp. was only isolated from freshly prepared yogurt and it highlights the fact that locally

prepared fresh yogurt without ice is safe to consume. Thus, environmental sanitation and personal hygiene still play important roles to reduce bacterial enteric infections. Standard pasteurization milk is always essential.

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