

---

**CONTENTS**

Evaluation of results on present conventional and alternative vector control activities in prevention and control of dengue/dengue haemorrhagic fever (DHF) .....	65
<i>Thaung Hlaing, W. Tun Lin, Pe Than Htun, Sein Min, Sein Thaung, Htin Zaw Soe, Saw Lwin &amp; Soe Thein</i>	
Relationship between ABO blood groups and the HBsAg positivity in blood donors .....	74
<i>Win Aung, Sandar Nyunt, Khin Khin Aye &amp; Aung Thu</i>	
Efficacy and safety of artemisinin-piperazine (Artequick) compared to dihydroartemisinin-piperazine (Artekin) in uncomplicated falciparum malaria in adults .....	78
<i>Khin Phyu Pyar, Win Win Myint, Myat Phone Kyaw, Thaw Zin &amp; Marlar Than</i>	
Hepatitis B surface antigen sero-prevalence in two border towns near Thailand .....	83
<i>Khin May Oo, Yi Yi Kyaw, Ohmar Lwin, Aye Aye Yee, San San Oo, Khine Win, Myo Khin &amp; Khin Pyone Kyi</i>	
Utilization of basic health staff by rural community in disease management of malaria.....	88
<i>Hla Soe Tint, Myitzu Tin Oung, Thein Tun &amp; Thar Tun Kyaw</i>	
Lead contamination of common Myanmar foodstuffs prepared/stored in ceramic glazed potteries .....	93
<i>Phyu Phyu Aung, Aye Aye Than, Theingi Thwin, Khine Khine Lwin, Thidar Khine, Khin Taryar Myint &amp; Khin Myat Tun</i>	
Predictors of defaulting from anti-tuberculosis treatment in selected townships of Upper Myanmar .....	98
<i>Hla Soe Tint, Myitzu Tin Oung &amp; Bo Myint</i>	
Breast milk thiamine level of mothers with infants, clinically diagnosed as beri beri .....	104
<i>Than Nu Shwe, Thein Thein Myint, Aye Maung Han, Aye Thaung, Theingi Thwin, Phyu Phyu Aung, Moh Moh Hlaing, Moe Thida Kyaw &amp; Khin Myat Tun</i>	
Characterization of <i>Neisseria gonorrhoeae</i> strains isolated from patients attending the Sexually Transmitted Diseases (STD) and gynaecology clinics in Yangon .....	110
<i>Wah Wah Aung &amp; Kyi Kyi Thinn</i>	

Short report: Traditional beliefs and practices relating to chest symptoms among Shan people in a rural area of PyinOoLwin Township ..... 117  
*Hla Soe Tint, Tin Tin Wynn, Kyaw Kyaw, Thida, Thidar Htwe Win, Myitzu Tin Oung, Zaw Win Tun & Kyaw Zin Thant*

Short report: Estimation of disease burden due to tuberculosis (TB) in Insein Township, Yangon ..... 119  
*Yin Thet Nu Oo, Myo Myo Mon, Ko Ko Zaw, Kyaw Oo, Thandar Lwin & Saw Saw*

.....

**Editor-in-Chief**

Dr. Khin Pyone Kyi

---

**Editor**

Dr. Kyaw Min

**Associate Editors**

U Aung Myo Min

Dr. Ni Thet Oo

---

**Editorial Board**

Prof. Dr. Aye Maung Han

Dr. Tun Pe

Prof. Dr. U Kyaw

Dr. Than Tun Sein

Prof. Dr. Ne Win

Dr. Myo Khin

Prof. Dr. Myo Win

Dr. Khin Myat Tun

Prof. Dr. Kyi Kyi Thinn

Dr. Kyaw Moe

Prof. Dr. Myo Oo

Dr. Thaw Zin

Prof. Col. Thein Myint Thu

Dr. Khin Nwe Oo

Prof. Dr. Soe Aung

Dr. San Shwe

Dr. W.Tun Lin

Dr. Kyaw Zin Thant

---

**Business Manager**

Dr. Ye Htut

**Editorial Manager**

U Htain Win

**Production Manager**

U Ye Thway

Daw Win Win San

---

Printed at the Publication Division, Department of Medical Research (Lower Myanmar)

---

Restricted for Internal Use Only

**Evaluation of results on present conventional and alternative vector control activities in prevention and control of Dengue/Dengue Haemorrhagic Fever (DHF)**

*\*Thaung Hlaing, \*\*W. Tun Lin, \*Pe Than Htun, \*Sein Min, \*Sein Thaung, \*\*\*Htin Zaw Soe, \*\*\*\*Saw Lwin & \*\*Soe Thein*

\*Medical Entomology Research Division

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

\*\*\*Department of Medical Research (Central Myanmar)

\*\*\*\*Department of Health

A total of 395 and 243 houses in two selected wards of Kyimyindine and Tamway townships, respectively were surveyed for *Aedes aegypti* adults and larvae to obtain baseline entomological indices. Establishment of innovative larval control methods has been achieved during pilot surveys and laboratory experiments were successfully undertaken before the intervention. Even during the hot dry season, more than 50% of the total houses examined were found to be positive for larvae in both townships. During pre-intervention surveys, all entomological indices increased after the onset of rain. A training workshop to transfer the innovative larval control methods to the local community was held at Kyimyindine (intervention) Township. Close supervision and appropriate larval control methods were introduced in the intervention township. The methods included periodic augmentative release of larvivorous fish and dragon-fly nymphs, and use of cotton net sweepers. Post-intervention evaluations were undertaken twice per month. All indices gradually decreased to a very low level in intervention ward (Breteau Index dropped from 201 to 62). At the same time, the indices remained unchanged or were slightly above pre-intervention levels in non-intervention ward of Tamway Township (Breteau Index varied from 100 to 127). There was also a significant reduction of key premises ( $\chi^2= 14.49, p<0.0001$ ) and key containers ( $\chi^2= 25.49, p<0.0001$ ) in intervention ward. Regarding dengue morbidity, decrease in both clinically suspected dengue and reported DHF cases were evident at the intervention township. It showed that regular close-monitoring with active community participation is effective and is essential for prevention and control of dengue/DHF.

## INTRODUCTION

Dengue Fever/Dengue Haemorrhagic Fever (DF/DHF) is the most important mosquito-borne viral disease in the world in terms of morbidity, mortality and economic cost, with over 100 million cases per year. The global resurgence of DF/DHF has been threatening more than 2.5-3 billion people in over 100 countries worldwide. Methods for controlling or preventing dengue mainly rely on combating the mosquito vectors. In the light of the rising problem, it is important to activate the community-based

alternative vector control strategies. Selective and integrated mosquito vector control with community and inter-sectoral participation was proposed by the World Health Organization in 1995 as one of the five major components of the global strategy for dengue prevention and control in the regions [1]. The Tropical Diseases Research Programme (TDR) from the World Health Organization also encouraged the development and evaluation of community-based mosquito control strategies and scaling-up of successful pilot community-based interventions.

Dengue Fever (DF)/DHF has become an increasing public health problem in South-east Asia region including Myanmar, facing complex emergency outbreaks situations frequently. It is endemic in Myanmar with a 3-4 year epidemic cycle. The average number of cases and deaths per year for the whole country were 2537 and 107 during 1970 to 1986 [2]. The incidence has been increasing over the past 2-3 decades and upward trend is still continuing [3]. The trend is also more or less similar in succeeding years up to the present period.

There are limitations in present control measures such as changing water, sieving or tipping and covering water containers, spraying houses around 100 yards radius from hospitalized DHF cases. An increasing effort is being made to control the vector through community participation by reducing larval habitats, but densities still remain very high [4]. The major vector, *Aedes aegypti*, breeds primarily in man-made containers such as domestic water storage tanks, metal drums, cans, earthen pots, used tyres and other items that retain water including natural habitats. Although public health measures for source reduction are being undertaken, there still remains to develop efficient larval control programmes using all locally available vector control methods (such as biological, chemical and mechanical means), which should include community awareness and full participation. There is a need to upgrade alternative vector control activities targeted against *Aedes* larvae and adults with active community participation.

Categorization of containers (major, minor and miscellaneous) by types and quantitative determination of larval abundance by visual estimates have already been developed and tested by Department of Medical Research, Lower Myanmar (DMR-LM). It is important to determine the prevalence of *Aedes aegypti* larvae positive houses/containers, key premises and key containers by proper sampling. Missing one key container could be equivalent to missing

1,000 flower vases containing *Aedes aegypti* larvae [5]. Our study was carried out with the aim for evaluating dengue/DHF prevention and control activities based on appropriate integrated cost-effective vector control methods with emphasis on targeting key premises/key containers and sustained active community participation for reduction of DHF in Myanmar.

#### *General objective*

- To determine the effectiveness of alternative vector control methods in comparison to the present conventional control methods with a view to develop a sustainable selective and integrated vector control model for reduction of morbidity and mortality of DHF in Myanmar

#### *Specific objectives*

- To determine the prevalence of key premises/key containers at the two selected wards of two sub-urban townships in Yangon Division before and after intervention
- To compare selected entomological indices regarding *Aedes aegypti* larval and adult mosquito densities at these two selected wards
- To critically assess and reinforce the current vector control activities with closely supervised control methods in the intervention township

## **MATERIALS AND METHODS**

#### *Study design*

The study was a quasi-experimental field intervention study with non-randomized control, starting from April 2003 to October 2003. The actual planning and standardization and establishment of laboratory and field methods were accomplished one year prior to the starting date. Pre and post-intervention indicators were House Index (HI=the percentage of houses that have larvae in at least some positive containers), Container Index (CI=the percentage of

larvae positive containers), Breteau Index (BI=number of positive containers per 100 houses inspected) and adult mosquito density (by ten minutes aspirator catching per house).

#### *Study area*

Thar-Du and Ka-Ga wards from Kyimyindine and Tamway townships, respectively with high endemic DHF cases in sub-urban area of Yangon Division were selected purposively to ensure that the socio-economic situation, housing condition, container categories/types and the composition of under 15 years population of two wards are as similar as possible. Out of 27 wards in Kyimyindine Township, Thar-Du ward (east and west) comprised of 469 houses of which 30% were partitioned (into housing units) to accommodate usually two to four persons, and sometimes up to twelve families. Altogether there were 1,135 families, making a total population of 5,233 including 2,496 under 15-year of age. Most houses (60%) were double-storey and the rest were single or high-rise buildings. Each family has water for domestic use in its own containers. There were three community tanks (two covered and one uncovered). Thar-Du was the intervention ward using appropriate larval control measures with close-supervised monitoring and community participation for comparison with routine control measures. Out of 20 wards in Tamway Township, Ka-Ga ward comprised of 606 houses of which 38% were partitioned into two to fourteen housing units. Altogether there were 1,473 families, making a total population of 6,404 including 2,531 under 15-year of age. There were also three (covered) community tanks.

#### *Study methods*

A total of 395 and 243 houses in Thar-Du and Ka-Ga wards, respectively were surveyed for *Aedes aegypti* adults and larvae to obtain baseline entomological indices. The number of houses inspected was based on the expected level of infestation and the desired level of confidence in the results.

A total number of nearly 10,000 housing in each study township, the expected House Index was 50% (preliminary data) and a 95% confidence interval of 44%-56%, it would be necessary to inspect 300 houses [1]. Establishment of innovative larval control methods has been achieved as pilot surveys and laboratory experiments were being undertaken. The methods included periodic augmentative release of larvivorous fish [6, 7] and dragon-fly nymphs [8], and the use of cotton net sweeper [9, 10]. Ethical consideration was reviewed and approved by Ethical Review Committee, DMR-LM.

Community motivation, supervision and technology transfer: A training workshop to transfer the larval control methods to the local community was held at Kyimyindine (intervention) Township. Close-supervision and appropriate larval control methods were introduced in intervention township by Medical Entomology Research Team from DMR-LM in collaboration with health personnel from Department of Health, local authorities and non-governmental organizations such as Myanmar Maternal and Child Welfare Association, Myanmar Red Cross Association and Auxiliary Fire Brigade. Locally developed cotton net sweepers together with instruction for use were distributed to all wards of Kyimyindine Township.

Surveys: There were altogether ten surveys including three pre-intervention (during April and May 2003) and seven post-intervention evaluations (June-September, 2003). Post-intervention surveys were undertaken bi-weekly. Activities included inspecting and counting domestic water storage containers, positive *Aedes* larval estimation by visual method [10] and catching adult mosquitoes by aspirator. Every alternate house (i.e., 200 households in Kyimyindine Township and 135 households in Tamway Township) was selected for evaluations 3 to 7 based on the preliminary findings of more than 50% larvae positive of the total houses examined

in both townships even during the hot dry season (Surveys 1 and 2). Evaluations 8 and 9 in Kyimyindine and 8<sup>th</sup> survey in Tamway were inspections of the remaining alternate houses so that actual larval situation from houses that were not sampled previously could be ascertained from two wards. The final two evaluations included complete inspection of all houses as that of surveys 1 and 2 (a total of 394 and 243 houses in Thar-Du and Ka-Ga wards, respectively).

Close monitoring and intervention (Biological control): During post-intervention evaluations, the appropriate larval control methods were implemented to reduce larval populations in Thar-Du ward. The local larvivorous fish and dragon-fly nymphs were released in positive and key containers during every post-intervention survey with the help of the local community. Dragon-fly nymphs (pond variety) *Crocothemis servilia* (Drury) were collected from Kandawmin Lake near DMR-LM and larvivorous fish (*Aplocheilus panchax*) were caught from natural water bodies such as pond and creeks in Hlinethaya Township, the outskirt of Yangon City.

Close monitoring and intervention (Mechanical control): Dipping with cotton net sweeper in larvae positive metal drums was undertaken by the local community weekly with the supervision of the research team especially in the first two surveys.

*Aedes aegypti* adult mosquito surveillance: Ten minutes catching mosquito with aspirator [11] was performed on 30 randomly selected houses in each ward once before and two times after intervention period. Using two aspirators, two staff spent three hours collecting adult mosquitoes at Thar-Du ward of Kyimyindine Township (visiting 15 houses each between 8:30 and 11:30 hours local time), and then on the following day at Ka-Ga ward of Tamway Township. Indoor density of *Aedes aegypti* female adult was determined by ten minutes aspirator catching per house.

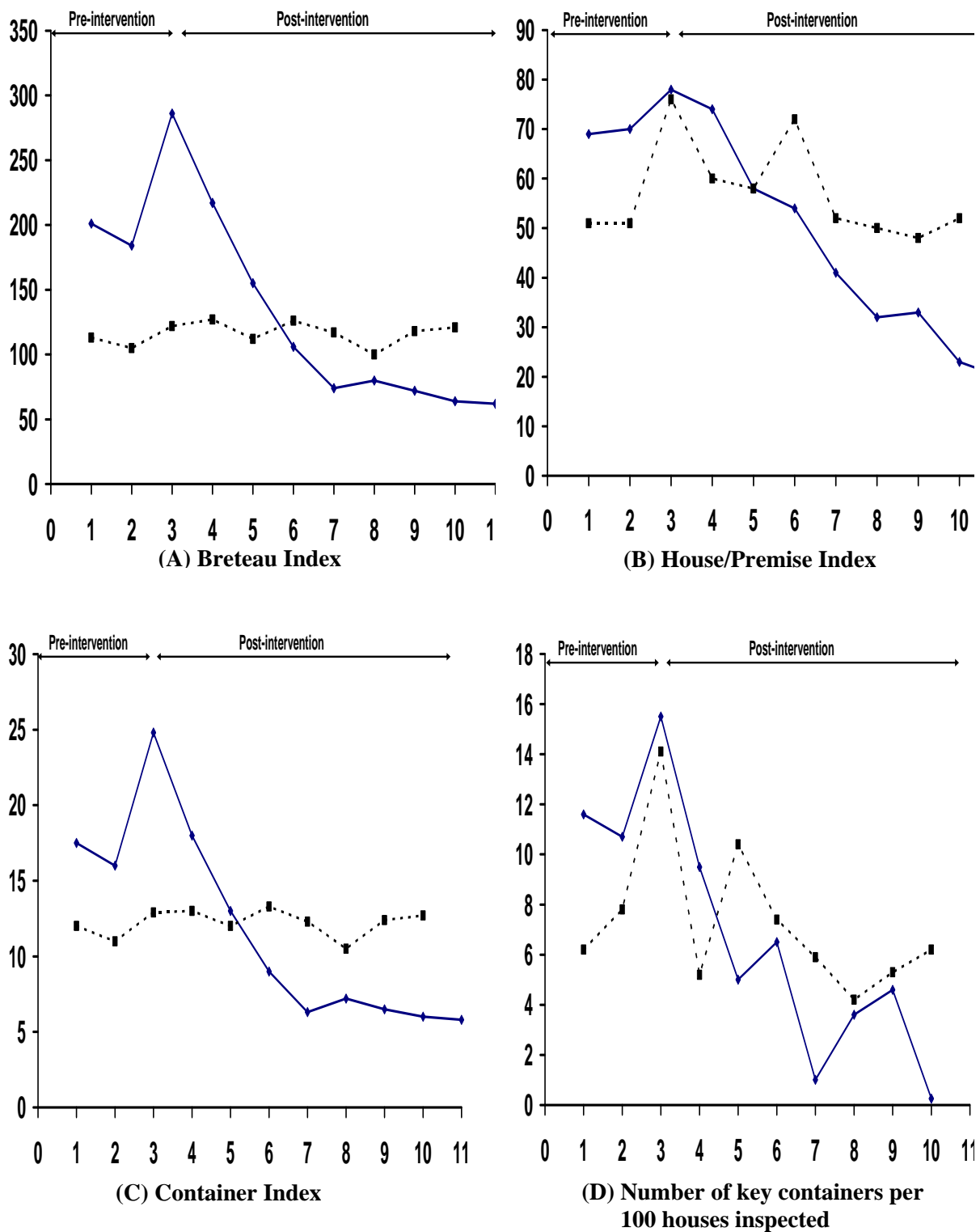
#### *Instrument for data collection, processing and analysis*

Household/family units, domestic water containers examined and *Aedes* larvae positive containers were recorded using separate survey forms. Cotton net sweepers and aspirators were used for larvae and mosquito adult collections, respectively. Data entry, sorting and data analysis were carried out using Microsoft excel and STATA. Chi-square tests for bivariate analysis and Poisson regression for group data were used for multivariate analysis. 'Z' test" was used for detection of differences in two proportions (rates).

## RESULTS

Even during the hot dry season, more than 50% of the total houses examined were found to be positive for larvae in both townships (Table 1). In pre-intervention surveys (surveys 1 to 3), all entomological indices increased due to the onset of rain. Post-intervention surveys (evaluations 4 to 10) were undertaken twice a month and all indices gradually decreased to a very low level in Thar-Du ward while they remained unchanged or slightly above pre-intervention levels in Ka-Ga ward (Fig. 1-A, B & C). A sustained reduction was only achieved after several evaluations by undertaking repeated supervision and close-monitoring in Thar-Du ward. By adjusted sequence of measures and time, using Poisson regression model, the prevalence rate ratio of the two townships was 0.87 ( $p < 0.0005$ ). In other words, the prevalence of finding larvae positive houses in Thar-Du ward of Kyimyindine Township was significantly reduced.

It was clearly evident that more than half (63% in Thar-Du ward of Kyimyindine and 55% in Ka-Ga ward of Tamway townships) of positive containers were produced by only 15% of the total houses (key premises). This is also true for key containers as the majority of the key containers detected were from key premises, 33 key containers (80%)



Number of surveys

Fig. 1. *Aedes aegypti* larval density indices and number of key containers per 100 houses inspected in Thar-Du ward, Kyimyindine Township (unbroken lines) and Ka-Ga ward, Tamway Township (broken lines) during the study period, April-October 2003.

Table 1. Total number of houses and containers harbouring *Aedes aegypti* larvae in Thar-Du ward of Kyimyindine (intervention) Township and Ka-Ga ward of Tamway (non-intervention) Township, Yangon, April and October 2003

Evaluation (2 wks interval)	No. of houses inspected	No. of houses with <i>Aedes</i> larvae positive (%)	No. of containers inspected	No. of <i>Aedes</i> larvae positive containers	BI
<i>Kyimyindine</i>					
<i>Before*</i>					
1-April	395	273 (69)	4549	795	201
2-May	394	276 (70)	4538	725	184
3-May	200	156 (78)	2305	572	286
<i>After*</i>					
4-June	200	148 (74)	2406	434	217
5-July	200	116 (58)	2355	310	155
6-July	200	108 (54)	2355	212	106
7-Aug	200	82 (41)	2358	148	74
8-Aug	195	62 (32)	2155	156	80
9-Sept	195	64 (33)	2155	140	72
10-Sept	394	89 (23)	4228	252	64
11-Oct	394	80 (20)	4228	244	62
<i>Tamway</i>					
<i>Before*</i>					
1-April	243	124 (51)	2289	275	113
2-May	243	124 (51)	2311	255	105
3-May	135	103 (76)	1280	165	122
<i>After*</i>					
4-June	135	81 (60)	1280	171	127
5-July	135	78 (58)	1280	151	112
6-July	135	97 (72)	1280	170	126
7-Aug	135	70 (52)	1280	158	117
8-Aug	120	60 (50)	1140	120	100
9-Sept	243	117 (48)	2308	287	118
10-Oct	243	126 (52)	2308	294	121

\*Period in relation to beginning of close monitoring and supervised vector control activities in Thar-Du ward of Kyimyindine Township

BI = Breteau Index (Number of positive containers per 100 houses inspected)

in Thar-Du ward of Kyimyindine Township and 9 key containers (60%) in Ka-Ga ward of Tamway Township. The number of *Aedes aegypti* larvae positive houses with one or more positive containers in April and October 2003 (evaluations 1 and 10) is shown in Table 2. Defining household as a denominator, the proportion of finding larvae positive house between the two wards was 0.87:1 with 95% confidence interval of 0.86-0.88. Adjusted "before and after effect", total larvae positive houses as well as key

Table 2. Total number of *Aedes aegypti* larvae positive houses in Thar-Du ward of Kyimyindine (intervention) Township and Ka-Ga ward of Tamway (non-intervention) Township, Yangon, April and October 2003

No. of houses examined	No. of larvae positive houses (%)	Houses with no. of container positive			No. of larvae negative houses (%)	
		One (%)	Two (%)	Three & above (%)		
<i>Kyimyindine (KMD)</i>						
Before	395	273 (69)	133 (34)	80 (20)	60 (15)	122 (31)
After	394	80 (20)	38 (10)	30 (7)	12 (3)	314 (80)
<i>Tamway (TME)</i>						
Before	243	124 (51)	62 (26)	31 (13)	31 (13)	119 (49)
After	243	126 (52)	68 (28)	30 (12)	28 (12)	117 (48)
<i>Prevalence rate ratio (adjusted before and after effect)</i>						
KMD:TME	0.87: 1	0.81: 1	1.11: 1	0.75: 1		
(95% confidence interval)	(0.86 - 0.88)	(0.8 - 0.82)	(1.09 - 1.13)	(0.74 - 0.77)		

premises were significantly lowered in Thar-Du ward than in Ka-Ga ward. There was a significant reduction of total positive containers ( $\chi^2=50.06$ ,  $p<0.0001$ ), major positive containers ( $\chi^2=26.06$ ,  $p<0.0001$ ) and key containers ( $\chi^2=25.49$ ,  $p<0.0001$ ) in Thar-Du ward. Also, 25% - 50% reduction of key premises was evident in Thar-Du (intervention) ward ( $\chi^2=14.49$ ,  $p<0.0001$ ) (Table 3).

Regarding *Aedes aegypti* adult density in study areas, there was also a 50% reduction in mosquito population of intervention ward and was statistically highly significant ( $\chi^2=10.45$ ,  $p=0.001$ ).

## DISCUSSION

Our study was a quasi-experimental field intervention study. The main aim was to evaluate and reinforce the present conventional dengue/DHF prevention and control activities using integrated vector control. Special emphasis was given on targeting key premises (15%) and key containers (60-80%) by using appropriate larval control methods such as bio-control (locally available larvivorous fish and dragon-



Table 3. Total number of *Aedes aegypti* larvae positive different container categories and number of key containers before and after intervention in Thar-Du ward of Kyimyindine Township and Ka-Ga ward of Tamway Township, Yangon, April and October 2003

Container categories	No. of containers examined	No. of larvae positive containers	Key containers	Prevalence rate ratio *
<i>Kyimyindine</i>				
Before (1 <sup>st</sup> survey)				
C 1	1089	196	27	<u>C 1 (Positive)</u> 0.47
C 2	3337	568	17	(0.46-0.48)
C 3	123	31	2	
Total	4549	795 (17.5%)	46	<u>C 1 (Key)</u> 0.52
After (10 <sup>th</sup> survey)				
C 1	1032	80	1	(0.51-0.54)
C 2	3174	161	0	<u>C 2 (Positive)</u>
C 3	22	3	0	1.27
Total	4228	244 (5.8%)	1	(1.26-1.27)
<i>Tamway</i>				
Before (1 <sup>st</sup> survey)				
C 1	408	110	11	<u>C 2 (Key)</u> 0.95
C 2	1839	152	4	(0.93-0.96)
C 3	42	13	0	<u>C 3 (Positive)</u>
Total	2289	275 (12%)	15	0.76
After (10 <sup>th</sup> survey)				
C 1	427	116	9	(0.7-0.83)
C 2	1855	170	6	<u>C 3 (Key)</u>
C 3	26	8	0	Not relevant
Total	2308	294 (12.7%)	15	

\* = Adjusted before and after effect (95% Confidence interval)

C1 = Major container category (e.g. metal drums, barrels, ceramic tanks)

C2 = Minor container category (e.g. earthen pots and small jars, flower vase)

C3 = Miscellaneous (e.g. discarded old batteries, broken pots, plastic bottles, car tyres)

fly nymphs) and mechanical control (using locally developed cotton net sweepers).

The predatory rates, catching, transportation and release of bio-control agents into positive containers, and efficient larval sweeping were successfully carried out as have been in previous studies [6, 7, 8]. The mean percent removal of *Aedes aegypti* larvae per drum was  $85.36\% \pm 10.74$  and it was found to be a simple and cost-effective mechanical control method that was readily accepted by the community. A pilot field study, involving periodic augmentative

release of dragon-fly nymphs performed by Sebastian *et al.* [8] was an excellent work and this technology was used in this study showing that sustained bio-control could be achieved with the awareness and enthusiastic participation of local householders in Yangon. Larvivorous fish (*Aplocheilus panchax*) was also an appropriate, simple bio-control technology since this local fish is available in peri-urban areas of Yangon.

Baseline entomological indices in pilot surveys were found to be higher than DOH annual reports. To assess the levels of *Aedes aegypti* infestations, we applied the most common three larval indices, mainly HI, BI and CI. Actually, Breteau Index (BI) establishes a relationship between positive containers and houses, and is considered to be the most informative and relevant for focusing control efforts on the management or elimination of the most common habitats [1]. *Aedes aegypti* adult mosquito density was monitored using a locally developed aspirator (ten minutes catching per house) instead of using human as baits for mosquito collection in the past.

In our study, about one thousand larvivorous fish and three hundred dragon-fly nymphs were utilized. Approximately 70% of households accepted the introduction of these fish whereas about 30% of the households utilized the dragon-fly nymphs. Some of the local drums and cement tanks already have the dragon-fly nymphs (container variety) *Bradinopyga geminata* (Rambur) and the community seeded these nymphs into other major containers that do not contain these nymphs. Naturally, before the intervention, it showed that more than half of total positive and key containers were from key premises and the majority was from major container categories. This would be concluded that it is essential to detect key premises (premises with at least three positive containers for *Aedes aegypti* larvae) and key containers (containers that have at least 500 larvae in each container) that are producing high numbers of dengue vectors. Proper mapping of high risk areas

(hot spots) and reducing child vector contact are of paramount importance. Detection of key containers near areas where children congregate during the daytime must be taken care of immediately.

Dengue is a worldwide problem but a common approach is a realistic thing [12]. Emphasis should be on the development of effective sustainable control programmes based on source reduction using community participation [13, 14].

Myanmar has a strong local NGOs and this should be exploited for mapping, detection and elimination of key containers under the supervision of the local township administrative and health authorities [10]. The present results demonstrate the feasibility and effectiveness of close supervision, community motivation and active participation. Community participation in various levels such as households, school and day-care centres etc. has been achieved and dengue vector densities have been reduced in a sustainable manner during the study period. There is a potential benefit for children of <15 years in intervention ward. It is recommended that proper mapping and concentrating on key premises and key containers and utilizing appropriate larval control methods are the critical success factors and should be encouraged utilizing active community participation. More health education on source reduction should be given to the health staff and the community including NGOs than at present.

Although both larval and adult indices gradually decreased to a very low level in intervention ward, a sustained reduction was only achieved after several evaluations. The first two surveys were pilot studies. From third to seventh surveys, every alternate house (200 and 135 households in Kyimyindine and Tamway townships, respectively) was inspected based on the preliminary findings that more than 50% of the houses were positive for larvae in both townships. More repeated supervision and close monitoring is needed for sustainability of the innovative vector control measures.

If the study could have been undertaken on more than two townships using paired randomization, the results would have been more convincing.

## ACKNOWLEDGEMENT

We are grateful to our former Director-General, DMR-LM, H.E. Deputy Minister, Professor Dr. Paing Soe for his permission and support to conduct the study, the township medical officers and staff of Kyimyindine and Tamway townships for their help and cooperation, staff of Medical Entomology Research Division for the assistance rendered, the ward elders, field staff and the residents of Kyimyindine and Tamway townships for their active participation in our intervention surveys. Thanks are also due to Dr. Mon Mon and Dr Kyaw Oo for their statistical advice and U Tin Maung Maung for his computing assistance. This study was funded by WHO/APW.

## REFERENCES

1. WHO Comprehensive Guidelines. Prevention and Control of Dengue and Dengue Haemorrhagic Fever. *Regional Publications* 1999; SEARO No. 29.
2. Soe Thein. Dengue Haemorrhagic Fever in Myanmar. *DMR Bulletin* 1991; 5(3), 86-9.
3. Khin Mon Mon, Saw Lwin, Soe Aung & Thar Tun Kyaw. Epidemiology of Dengue Haemorrhagic Fever in Myanmar, 1991-1998. *Dengue Bulletin* 1998; 22: 49-52.
4. Prasittisuk C, Andjaparidze AG & Kumar V. Current Status of Dengue/Dengue Haemorrhagic Fever in WHO Southeast Asia Region. *Dengue Bulletin* 1998; 22, 1-11.
5. Tun Lin W, Kay BH & Barnes A. Understanding productivity, a key to *Aedes aegypti* surveillance. *American Journal of Tropical Medicine and Hygiene* 1995; 53(6): 595-601.
6. Htay Aung, Myo Paing & Lwin Lwin Oo. The efficacy of mosquito fish *Trichogaster trichopterus* for control of *Aedes aegypti* in a community, Yangon. *Myanmar Health Sciences Research Journal* 1991; 3(1), 36-9.
7. Tun Lin W, Sein Min, Thaug Hlaing, Nyan Sint, Pe Than Htun & Mg Mg Mya. The use of local larvivorous fish: an appropriate simple

- technology for malaria control in south eastern part of coastal Myanmar. *Abstracts of Posters, Myanmar Health Research Congress 2000*; 47.
8. Sebastian A, Myint Myint Sein, Myat Myat Thu & Corbet PS. Suppression of *Aedes aegypti* (Diptera: Culicidae) using augmentative release of dragonfly larvae (Odonata: Libellulidae) with community participation in Yangon, Myanmar. *Bulletin of Entomological Research* 1990; 80, 223-232.
  9. Tun Lin W, Maung Maung Mya, Sein Maung Than & Tin Maung Maung. Rapid and efficient removal of immature *Aedes aegypti* in metal drums by sweep net and modified sweeping method. *Southeast Asian Journal of Tropical Medicine and Public Health* 1995; 26(4):754-9.
  10. Tun Lin W, Soe Aung, Myint Lwin *et al.* Detection of major breeding sources for the Dengue Haemorrhagic Fever vector *Aedes aegypti* in Yangon city. *Abstracts of Papers, Myanmar Health Research Congress 1996*; 56.
  11. Thaug Hlaing, Tun Lin W, Sein Thaug *et al.* Development and testing of a locally made adult mosquito collecting aspirator for prevention and control of Dengue and Dengue Haemorrhagic Fever (DHF) in Myanmar. *Abstracts of Papers, Myanmar Health Research Congress 2002*; 53.
  12. Reiter P. Status of current *Aedes aegypti* control methodologies. In: *Dengue a worldwide problem, a common strategy*. Halstead SB, Gomez-Dantes H (eds), Ministry of Health, Mexico and Rockefeller Foundation, NY, 1992; 41-8.
  13. Service MW. Importance of ecology in *Aedes aegypti* control. *South-east Asian Journal of Tropical Medicine and Public Health* 1992; 23: 681-9.
  14. Gubler DJ & Clark GG. Community-based integrated control of *Aedes aegypti*: A brief overview of current programmes. *American Journal of Tropical Medicine and Hygiene* 1994; 50: 50-60.

## Relationship between ABO blood groups and the HBsAg positivity in blood donors

*\*Win Aung, \*Sandar Nyunt, \*Khin Khin Aye & \*\*Aung Thu*

\*Blood Programming Division

\*\*Clinical Research Division

Department of Medical Research (Lower Myanmar)

Prior to the production of plasma-derived hepatitis B vaccine, HBsAg-positive blood units which had been collected from various blood banks of hospitals in Yangon area were tested again for confirmation and determination of the degree of HBsAg positivity by using the Counter Current Electrophoresis (CEP) method at the Hepatitis B Vaccine Plant, DMR (LM). Only those blood units with high titer of HBsAg positivity (i.e. titer  $\geq 1/10$  dilution) were used for further production processes. In this study, ABO blood groups distribution in 6505 HBsAg-positive blood donors with low and high titers positivity of HBsAg were determined. It was found that ABO blood groups were equally distributed in HBsAg-positive blood donors. However, high titer of HBsAg positivity was apparently encountered in those donors with blood group AB compared to other blood groups. Donors with blood group AB were found to be more susceptible than others to have high titre positivity of HBsAg.

### INTRODUCTION

Hepatitis B (HB) viral infection is a major public health problem worldwide. The HB vaccination is an effective protective measure against the HB viral infection and the plasma-derived HB vaccine entered the global market in 1981 [1]. In Myanmar, HB viral infection is regarded as one of the vaccine preventable diseases taking 8th position among the prioritized list of diseases and health conditions in National Health Plan (2006-2011) [2]. The plasma-derived HB vaccine was successfully developed by the Department of Medical Research, Lower Myanmar (DMR, LM) and has been distributed to the public since 1997 [3]. Prior to the production of plasma-derived hepatitis B vaccine, hepatitis B surface antigen (HBsAg) positive blood units collected from various blood banks of hospitals in Yangon area were tested again for confirmation and determination of the degree of HBsAg positivity by using the Counter Current Electrophoresis (CEP)

method. Only those blood units with high titer of HBsAg positivity i.e. titer  $\geq 1/10$  dilution were used for further production processes. In this study, ABO blood groups distribution among the HBsAg-positive blood units collected from various blood banks and those blood units with high titre of HBsAg positivity were studied. This study was conducted with an aim to determine ABO blood groups distribution among HBsAg-positive blood donors, and to verify the relationship between ABO blood groups distribution and degree or titer of HBsAg positivity in the HBsAg-positive blood donors.

### MATERIALS AND METHODS

This is a cross-sectional study, carried out at the Hepatitis B Vaccine Plant, DMR (LM). The HBsAg- positive blood units which had been already determined by ELISA test system (ORTHO antibody to HBsAg, USA) with known blood ABO groups from various hospitals in Yangon area, majority is from

the National Blood Centre, Yangon General Hospital, were collected for production of plasma-derived hepatitis B vaccine at the HB Vaccine Plant, DMR (LM). These blood units were tested again for degree of HBsAg positivity by using a semi quantitative method, i.e. Counter Current Electrophoresis (CEP) technique and also for anti-HCV, anti-HIV and anti-syphilis by using specific methods. Among them, those blood units with HBsAg positivity titer of  $\geq 1/10$  dilution negativity for anti-HCV, anti-HIV and anti-syphilis were selected and pooled for further production processes [4].

*Determination of the degree of HBsAg positivity*

The CEP testing is based on electroendosmosis in which the HBsAg particles carrying a prevalent negative surface charge migrate toward the anode and anti-HBs immunoglobulins, raised in an experimental animal, with partial dissociation migrate toward the cathode at pH 8.6 in veronal buffer in CEP tank. A clear precipitation line was formed between them and results were read immediately after run [5].

In this study, the titres of serum samples of HBsAg-positive blood donors were determined and categorized by CEP method after serial dilution i.e. 1 in 5, 1 in 10, 1 in 20, 1 in 40, etc. with 1% agarose in 0.015 M veronal buffer containing 5-10% normal human serum.

*Determination of the anti-HCV*

The detection of anti-HCV in serum of these donors was performed by using Serodia-HCV Gelatin Particle Agglutination Test, Fujirebio Inc. of Japan. The test is based on the principle that sensitized particles are agglutinated by the presence of antibodies to HCV in serum/plasma specimens.

*Determination of the anti-HIV*

The anti-HIV was determined by the 3rd generation one step antibodies to HIV-1/HIV-2 test, Standard Diagnostics, Inc. of the Republic of Korea. It is an immunochromatographic test for the qualitative detection

of antibodies of all types specific to HIV-1 and HIV-2.

*Determination of the anti-syphilis*

Syphilis antibodies were identified by the Syphilis RPR test, Human Biochemicals and Diagnostics, Germany. It is a macroscopic, non-treponomal flocculation test that is used to detect and quantify reagin (syphilis) antibodies in blood samples.

In this study, a total of 6505 HBsAg-positive blood units with known ABO blood groups were studied for their degree of HBsAg positivity; i.e. titer of low ( $< 1/10$ ) and high ( $\geq 1/10$ ) dilutions in these donors. Baseline distribution of ABO blood groups in Myanmar population of other studies were mentioned for comparison [6, 7, 8]. Student unpaired 't' test and 'Chi square' test were used for statistical analysis.

**RESULTS**

Percentage distribution of the ABO blood groups in 6505 HBsAg-positive blood donors of this study and previous studies in Myanmar population are shown in Table 1.

Table 1. Percentage distribution of the ABO blood groups in Myanmar population of various studies

	Total cases studied	No. of patients with different blood groups			
		A	B	AB	O
Mya Tu & Khin Maung Lwin, 1967	10035	2539 (25.3%)	31613 (31.5%)	833 (8.2%)	3502 (34.9%)
Soe Min Thein, 1978	6223	1575 (25.3%)	1837 (30.1%)	504 (8.1%)	2271 (36.5%)
Thi Wa Tin, 1992	27672	6863 (24.8%)	8772 (31.9%)	2324 (8.4%)	9713 (35.1%)
Present study	6505	1544 (23.7%)	2130 (32.7%)	504 (7.8%)	2327 (35.8%)

Percentage of cases studied are shown in parentheses

Percentage distributions of A, B, AB and O blood groups in present study were 23.7%, 32.7% , 7.8% and 35.8%, respectively and were not apparently different from those of previous studies. Therefore, our study on

6505 blood donors with HBsAg positivity was assumed to be valid.

Table 2 depicts age and sex distribution in donors with low and high titers of HBsAg positivity. There was no significant difference in age and gender distribution in both low and high titers of HBsAg positivity.

Table 2. Age and sex distribution in donors with low and high titers of HBsAg positivity

	Degree of HBsAg positivity		Significance 'p' value
	Low titer (n=5342)	High titer (n=1164)	
Age (years)	27±4.86	30±1.85	>0.05 *
X± SE (range)	(18-45)	(18- 54)	(NS)
Sex	4578 : 763	970: 194	>0.05 **
Male : Female	(6:1)	(5:1)	(NS)

\* Using Student's unpaired 't' test

\*\* Using 'Chi square' test

NS = not significant at the 5% probability level

Table 3 summarizes the percentage distribution of ABO blood groups in donors with low and high titers of HBsAg positivity. It was found that high HBsAg titers were encountered in 17.9% of 6505 blood units. The percentage distributions of ABO blood groups in those donors with high titre of HBsAg positivity were found to be 17%, 17.5%, 32.5% and 17.9% in blood group A, B, AB and O, respectively indicating that high titer of HBsAg positivity was significantly encountered in blood group AB compared to others.

Table 3. ABO blood groups distribution in the HBsAg-positive blood donors with low and high titers of HBsAg positivity

Degree of HBsAg positivity (by CEP method)	No. of HBsAg-positive blood donors with different blood groups				
	A	B	AB	O	Total
Low HBsAg titer (<1/10 dilution)	1282	1757	340	19628	5341
	83%	82.5%	67.5%	4.30%	82.1%
High HBsAg titer (≥1/10 dilution)	262	373	164	365	1164
	17%	17.5%	32.5%	15.7%	17.9%
Total	1544	2130	504	2327	6505

$$X^2 = 82.92$$

Degree of freedom = 3

P value = <0.001

## DISCUSSION

It is well documented that many diseases are associated with ABO blood groups; blood group A with higher incidence of cancer of stomach, pernicious anemia, and achlorhydria [9, 10], blood group O with duodenal ulcer and cholera [11, 12, 13.]. Regarding the hepatitis B viral infection, HB virus carrier i.e. HBsAg positivity were almost equally distributed in different ABO blood groups [14, 15] but Rhesus negative blood group donors were more susceptible than other to have HBsAg [16]. There have been some evidences of interaction between HB viral infection and blood cells [17, 18]. It is also a well known fact that higher degree of HBsAg positivity is usually associated with higher possibility of HBeAg, a marker of viral replication, and massive viral load leading to progressive and severe hepatic damage with poor prognosis.

In our study, regarding the prevalence of the HBsAg positivity among blood donors, it was found that the HBsAg positivity was almost equally distributed in different blood groups. Otherwise, there was no apparent association between frequency distribution of ABO blood groups and HBsAg carriers (Table 1). It is in accordance with the previous findings of Szmunn *et al.* [14] and Myo Aye *et al.* [15].

Regarding the degree of HBsAg positivity, age and gender factors were not related to the degree or titer of HBsAg positivity indicating that any age and sex have an equal chance of carrying HBsAg positivity and degree regardless of ABO blood groups (Table 2). Regarding the ABO blood groups with degree of HBsAg positivity, it is of interest to note that the HBsAg-positive donors with blood group AB were found to be more susceptible than other blood groups to carry HBsAg of high titer positivity. Otherwise, a person with blood group AB was found to be more likely to have high titer of HBsAg positivity than those with other blood groups (Table 3).

From the findings of our study, it could be concluded that HB viral infection followed by HBsAg positivity is not related to ABO blood groups and there was an equal chance of any person to carry HBsAg. Although a person with blood group AB also has an equal chance of being infected with HB viral infection, the possibility of high HBsAg titer was apparently found in donors with blood group AB compared to persons with other blood groups. Therefore, in clinical practice, persons with blood group AB should be more aware of HB infection prophylaxis than other persons and once being infected, they should take prompt and appropriate medical treatment to prevent further complications related to HB infection.

## REFERENCES

1. Maynard JE. Hepatitis B: global importance and need for control. *Vaccine* 1990; 8:18-20.
2. *Report of National Health Plan (NHP)*. Prioritized list of diseases and health conditions in National Health Plan (2006-2011).
3. Khin Pyone Kyi, Aye Kyaw, Myo Aye, Yi Yi Htwe, Khin May Oo, Than Aung *et al*. Human reactogenicity, safety and immunogenicity study of the hepatitis B vaccine produced at the Department of Medical Research. *Myanmar Health Research Congress*. Programme and Abstracts 1996; 3.
4. *Batch Processing Record*. Plasma Derived Hepatitis B Vaccine Production (35L), Hepatitis B Vaccine Plant, DMR (LM), 2003.
5. Prince AM & Burke K. Serum hepatitis antigen: rapid detection by high voltage immunoelectrophoresis. *Science* 1970; 169: 593-595.
6. Khin Maung Lwin & Mya Tu. Distribution of the ABO blood groups in various national races. In: *Handbook of Biological Data on Burma* 1967; 143.
7. Soe Min Thein. ABO blood groups and cancers in Burmese population. *M.Med.Sc (Physiology) Thesis*, Institute of Medicine 2, Yangon, 1978.
8. Thi Wa Tin. A study on ABO blood groups distribution in cholera patients in Infectious Diseases Hospital. *M.Med Sc (Thesis)*, Institute of Medicine (1). Yangon, 1992.
9. Aird J & Bentarl HH. A relationship between cancer of stomach and the ABO blood group. *British Medical Journal* 1953; 1:799-861.
10. Hartmann O & Steven P. ABO blood groups and cancer. *Lancet* 1964; 1: 1305-1306.
11. Vogal F. Controversy in human genetics: ABO blood groups and diseases. *American Journal of Human Genetics* 1970; 22: 464-475.
12. Barua D & Paguio AS. ABO blood groups and cholera. *Annals of Human Biology* 1977; 4: 489-492.
13. Chaudhuri A. Cholera and blood groups. *Lancet* 1977; 2: 404-405.
14. Szmunness W, Prince AM & Cherubin CHE. Serum hepatitis antigen carrier rate relation to ABO blood groups. *British Medical Journal* 1971; 2: 198-199.
15. Myo Aye, Than Aung, Kyi Win, Lay Wai, Khin Maung Win & Khin Pyone Kyi. Hepatitis B carrier rate in different blood group of blood donors in Myanmar. Abstracts of paper, *Myanmar Medical Research Congress* 1991; Dec 17-20: 2.
16. Kiagno JS, Missingo H & Mzula E. Relation of blood groups in hepatitis B virus antigen carrier state. *East Africans Medical Journal* 1982; 59.
17. Campbell AN & Freedman MF. Final marrow aplasia associated with non A, non B Hepatitis. *British Medical Journal* 1983; 286: 1820.
18. Gordon-smith EC. Aplastic Anaemia: Etiology and clinical features. *Baillere's Clinical Haematology* 1989; 2 (1): 1-15.

**Efficacy and safety of artemisinin-piperaquine (Artequick) compared to dihydroartemisinin-piperaquine (Artekin) in uncomplicated falciparum malaria in adults**

\*Khin Phyu Pyar, \*Win Win Myint, \*\*Myat Phone Kyaw,  
\*\*Thaw Zin & \*Marlar Than

\*\*Clinical Research Unit (Malaria), No. 1 MH (1000 Bedded), Mingaladon  
\*\*Department of Medical Research (Lower Myanmar)

A hospital-based, randomized controlled study was done at No. 1 MH (1000 Bedded), Mingaladon, No. 1 MH (700 Bedded), PyinOoLwin and No. 9 MH (100 Bedded), Lashio, on a total of 64 uncomplicated falciparum malaria patients from January to September 2007, to determine the therapeutic efficacy, safety and tolerability of artemisinin-piperaquine (Artequick) tablet in comparison with dihydroartemisinin-piperaquine (Artekin) for the treatment of 30 uncomplicated falciparum malaria patients in adults. Artequick 2 tabs was given at 0 and 24 hours in one group and Artekin 2 tabs was given at 0, 6, 24 and 32 hours in the control group. Initial parasitaemia/ $\mu$ l were  $16471.88 \pm 38755.1$  and  $13528.9 \pm 1909.3$ . Fever clearance times (FCT) were  $43.23 \pm 17.35$  and  $12.4 \pm 13.1$  hours, and Parasite clearance times (PCT) were  $57.6 \pm 21.88$  and  $46.8 \pm 22.1$  hours, respectively, in Artequick and Artekin groups. In Artequick group, there were four late treatment failures (LTF) that adequate clinical and parasitological response (ACPR) was 94% compared to 100% ACPR in Artekin group. There were no serious side effects. Artequick is as safe as Artekin but with lower ACPR in this study. Further dose finding studies will be needed to establish its efficacy in the treatment of uncomplicated falciparum malaria in adults.

## INTRODUCTION

To combat the development and spread of resistance to drugs in *P. falciparum* infection, WHO has recommended the use of artemisinin combination therapy (ACT) and, if possible, the preparations should be formulated in a single tablet to enhance the compliance. Artequick (artemisinin-piperaquine) and Artekin (dihydroartemisinin-piperaquine) are among these artemisinin combination therapy. Clinical studies on the Artekin (dihydroartemisinin-piperaquine) have been carried out in several countries including Thailand, Vietnam, Cambodia and Myanmar and are reported to be safe and effective [1].

Artequick (artemisinin-piperaquine) is an improved fixed dose Artemisinin combination therapy. Phase I, II and III clinical

studies on Artequick have been completed and reported [2]. Primary evaluation of Artequick on 1025 malaria patients in clinical trial (phase II and III) at 7 hospitals in 4 countries indicated that it was well tolerated, highly efficacious, quick acting and with low toxicity. Over 6000 people treated with Artequick in Kampong Speu of Cambodia also showed that it was very well tolerated and with a low cost. This new excellent ACT is thus expected to enter the public sector in a large scale in the near future [2].

A hospital-based, randomized controlled study of Artekin compound tablet (40 mg dihydroartemisinin and 320 mg piperaquine phosphate, the dosage of 2 tabs given at 0, 6, 24 and 32 hours) comparing with Artequin™ (fixed dose combination pack of artesunate – mefloquine, 2 tablets daily for 3 days course)



was done at the Defense Services General Hospital (DSGH), Mingaladon in 2006. The sensitivity was 100% for this combination. Then, randomized control trial of Artekin and Larimal fixed dose combination (Blister pack of artesunate 50 mg and 153.1 mg amodiaquine (dosage of 2 tablets BD for 3 days) gave 100% sensitivity in 2007 at DSGH.

Hence, this study on the clinical efficacy and safety of a fixed dose combination tablet of Artequick® will provide useful information for future evidence-based guidelines in the treatment of falciparum malaria in adults in Myanmar.

#### *Aim*

- To find out the efficacy, safety and tolerability of Artequick (artemisinin-piperaquine) compound tablet in uncomplicated falciparum malaria in Myanmar

#### *Objectives*

- To measure the clinical and parasitological efficacy of Artequick in uncomplicated falciparum malaria
- To evaluate the frequency, severity and duration of adverse clinical, haematological, biochemical and ECG features

## **PATIENTS AND METHODS**

#### *Study design*

An open label, randomized hospital-based clinical study.

#### *Study sites*

Clinical Research Unit (Malaria), No.1 DSGH (1000 Bedded), Mingaladon, No. 1 MH (700 Bedded), PyinOoLwin and No.9 MH (100 Bedded), Lashio.

#### *Study period*

March 2007 to September 2007

Acute symptomatic uncomplicated falciparum malaria patients admitted to the above hospitals were recruited. Patients were categorized as symptomatic if they were febrile or with one of the following

symptoms: Feeling ill, headache, aches and pains, nausea or vomiting.

#### *Inclusion criteria*

- Both sexes
- The age group between 12-60 years
- Positive peripheral blood film for trophozoite forms of pure *Plasmodium falciparum* with the count ranging from 2000/μl up to 200,000/μl
- Axillary temperature  $\geq 37.5^{\circ}\text{C}$  or history of fever during the previous 24 hours
- Patients who have no evidence of severe and complicated falciparum malaria
- Patients who have not been treated with any artemisinin derivatives or mefloquine within the past 14 days
- Patients who are willing to give informed consent for treatment and able to remain hospitalized for 28 days

#### *Exclusion criteria*

- Presence of any feature of severe or complicated malaria
- Presence of mixed infection (e.g. vivax or malariae)
- Patients with fever due to causes other than malaria e.g. TB, etc.
- Patients with other concomitant diseases like diabetes mellitus, etc.
- Pregnant women in the first trimester of pregnancy
- Contraindications to antimalarial drugs

#### *Withdrawal criteria*

- Patient's request
- Any serious adverse effects to drugs
- Serious or repeated non-compliance with protocol specifications

#### *Sample size*

##### Artequick

Sixty-four adults (24 patients from No.1 MH Mingaladon, 20 patients from No.1 MH PyinOoLwin and 20 patients from No.9 MH Lashio)

##### Artekin

Thirty adults (10 patients each from above three hospitals)

### *Study drug*

Artequick<sup>®</sup> (fixed dose combination artemisinin-piperaquine, expiry date 07–2009, produced from Artepharm Co. Ltd, Guangzhou, China, Batch No. 20060801). Each tablet contains artemisinin (AMS) 62.5mg and piperaquine (PPQ) 375mg.

Dose=2 tabs at 0 hour and 2 tabs at 24 hours (Total dosage=4 tabs).

### *Control drug*

Artekin (compound dihydroartemisinin-piperaquine, produced from Holleykin Pharmaceutical Co. Ltd., Guangzhou, China, Batch No. 20030302). Each tablet contains 40 mg dihydroartemisinin and 320 mg piperaquine phosphate.

Dose=2 tabs at 0, 8, 24, 32 hours. (Total dosage=8 tabs)

### *Procedure*

Eligible patients were subjected to the following procedure.

Routine history taking, clinical examination and relevant investigations were done. Randomization was done by lottery procedure. Data were recorded in the standard proforma by the assigned medical officer, under the supervision of the medical specialist in charge of the trial.

### *Assessments*

#### *Clinical*

Symptoms review, adverse effect review (according to the check-list format), and physical examination were made on Day 0, 1, 2, 3, 4, 7, 14 and 28. Body temperatures were taken 4 hourly until normal for 24 hours, then daily up to Day 28.

#### *Parasitology*

Giemsa-stained thick and thin blood smears were examined by the trained technician and parasite counts performed 6 hourly until negative for 24 hours and daily up to 3 consecutive negatives, then weekly up to Day 14 whenever it was indicated (e.g., reappearance of fever).

### *Haematology*

Haemoglobin %, total and differential count were carried out on day 0, 3, 7, 14 and 28.

### *Biochemistry*

Blood urea and sugar were examined on day 0, 3, 7, 14 & 28.

### *ECG*

Haematology, biochemistry and ECG were monitored on day 0, 1, 3, 7, 14 & 28 to the patients admitted to DSGH only and not in other hospitals.

### *Drug tolerance and safety*

Frequency, severity and duration of adverse clinical, haematological, biochemical and therapeutic response were assessed according to WHO criteria as ETF, LTF, ACPR.

### *Definitions (WHO 2003)*

#### *Early treatment failure (ETF)*

- Development of danger signs or severe malaria on Day 1, Day 2, or Day 3 in the presence of parasitaemia.
- Parasitaemia on Day 2 higher than Day 0 count, irrespective of axillary temperature.
- Parasitaemia on Day 3, with axillary temperature  $\geq 37.5^{\circ}\text{C}$ .
- Parasitaemia on Day 3  $\geq 25\%$  of count on Day 0.

#### *Late treatment failure (LTF)*

It is divided into late clinical failure (LCF) and late parasitological failure (LPF).

##### *Late clinical failure (LCF)*

- Development of danger signs or severe malaria after Day 3 in the presence of parasitaemia, without previously meeting any of the criteria of early treatment failure
- Presence of parasitaemia and axillary temperature  $\geq 37.5^{\circ}\text{C}$  on any day from Day 4 to Day 28, without previously meeting any of the criteria of early treatment failure.

Late parasitological failure (LPF)

- Presence of parasitaemia on Day 28 and axillary temperature  $<37.5^{\circ}\text{C}$ , without previously meeting any of the criteria of early treatment failure or late clinical failure

Adequate clinical and parasitological response (ACPR)

- Absence of parasitaemia on Day 28, irrespective of axillary temperature, without previously meeting any of the criteria of early treatment failure, late clinical failure or late parasitological failure

Efficacy

- Indicators include ETF, LTF, ACPR

PCT (Parasite clearance time)

- The time from initiation of therapy to the first negative blood film that remained negative for 48 hours

% PC<sub>24h</sub> and % PC<sub>48h</sub>

- Percentage clearance of parasitaemia at 24 and 48 hours

FCT (Fever clearance time)

- The time from initiation of therapy to time the temperature reached normal ( $37^{\circ}\text{C}$ ) and remained so for 24 hours

*Retreatment of failure*

- ETF and LTF cases were retreated with standard antimalarial treatment (according to the national guidelines). All cases were recovered.

*Data analysis*

All data in the proforma were checked for completeness, errors and inconsistencies (by the assigned investigators) prior to entry of the raw data into the constructed data-base sheet (Microsoft Office Excel). Statistical analyses were done by computer using EPI-INFO software at CRU (Malaria), DSGH.

*Ethical considerations*

The protocol was submitted to Ethical Committee (Research & Development Committee), Ministry of Defence for

approval. Informed written consents were obtained from all the patients.

## RESULTS

There were four late treatment failure cases out of 64 cases completed to study with Artequick, but there was no treatment failure case in Artekin group, out of 30 cases studied.

Table 1. Baseline characteristics of two groups

Parameters	Regimens		<p>
	Artequick (n=64)	Artekin (n=30)	
Age (years)	28.14±10.3	32.5 ±11.3	0.898
Body weight (kg)	60.00± 4.9	53.9 ± 7.3	0.965
Height (ins)	174.53± 8.4	163.9 ± 7.9	0.128
Initial temp ( $^{\circ}\text{C}$ )	38.20 ± 1.4	38.2 ± 1.1	0.937
Initial parasitaemia/ $\mu\text{l}$ (range)	400-200,000	800-200,000	0.939

Plus-minus values are shown in mean±SD

Table 2. Response of fever and parasitaemia in two groups

Response	Regimen		<p>
	Artequick (n=64)	Artekin (n=30)	
Fever clearance time FCT (Hours)	43.23±17.35	12.4±13.1	0.088
Parasite clearance time PCT (Hours)	57.60±21.88	46.8±22.1	0.091

Data are shown in mean±SD

Table 3. Serial laboratory (biochemical) parameters in two groups

Parameters	Day 0	Day 7	Day 14	Day 21	Day 28
<i>Artequick</i>					
Blood urea (mg%)	26.5 ±7.2	23.0 ±7.8	23.6 ±5.0	23.0 ±2.6	21.0 ±5.0
Blood sugar (mg%)	102.0 ±10.2	98.0 ±6.9	95.9 ±5.6	95.2 ±4.5	95.3 ±7.8
<i>Artekin</i>					
Blood urea (mg%)	26.3 ±7.4	24.7 ±6.3	22.5 ±1.7	22.2 ±2.6	23.1 ±5.8
Blood sugar (mg%)	96.4 ±21.5	102.1 ±10.1	99.1 ±9.1	99.6 ±5.5	101.8 ±7.2

Data are shown in mean±SD

There were no adverse events like dizziness, headache, nausea, vomiting, palpitations, pruritus and abnormal ECG finding in two groups.

Table 4. Serial laboratory (haematological) parameters in two groups

Parameters	Day 0	Day 7	Day 14	Day 21	Day 28
<i>Artequick</i>					
Hb(g/dl)	11.3 ±1.7	12.8 ±1.4	11.7 ±1.1	12.0 ±1.4	11.7 ±1.4
Total WBC (x10 <sup>3</sup> /l)	5.0 ±1.2	5.6 ±0.8	4.9 ±0.9	5.0 ±1.1	5.3 ±1.0
Differential	62.6	65.0	64.3	64.8	63.6
Polymorph (%)	±3.3	±3.9	±2.3	±3.1	±3.1
Lymphocyte (%)	33.3 ±2.6	30.0 ±3.2	33.3 ±3.7	31.1 ±3.1	31.6 ±3.0
Monocyte (%)	1.9 ±0.5	3.0 ±0.5	2.0 ±0.6	2.2 ±0.6	2.1 ±0.4
Eosinophil (%)	2.1 ±0.4	2.0 ±0.5	1.96 ±0.5	1.9 ±0.6	1.9 ±0.6
<i>Artekin</i>					
Hb (g/dl)	10.8 ±2.7	10.4 ±2.1	11.3 ±1.6	11.5 ±1.5	11.6 ±1.4
Total WBC (x10 <sup>3</sup> /l)	4.7 ±0.9	4.7 ±0.7	5.0 ±0.7	5.0 ±1.5	5.1 ±1.0
Differential	63.2	63.1	62.0	63.4	64.0
polymorph (%)	±3.3	±3.1	±4.2	±3.3	±3.1
Lymphocyte (%)	32.3 ±3.7	33.3 ±2.9	33.3 ±3.9	32.3 ±3.4	31.8 ±3.0
Monocyte (%)	2.1 ±0.0	1.9 ±0.5	1.7 ±0.5	2.0 ±0.4	1.8 ±0.4
Eosinophil (%)	2.2 ±0.4	2.3 ±0.4	2.2 ±0.5	2.3 ±0.5	2.2 ±0.6

Data are shown in mean±SD

## DISCUSSION

Southeast Asia region has the most resistant malaria parasite in the world limiting the treatment option. It is generally accepted that to combat drug resistance, combination of antimalarial drugs that include an artemisinin derivative should be used and, if possible, these should be formulated in a single tablet. RCT trials of Artekin have been done in many countries and showed good efficacy and tolerability. Present study shows that the drug Artequick has 4 LTF cases, having 94% ACPR. None of our patients develops severe side effects. Clinical trials of Artequick in Thailand [6] reported the cure rate of 72% at the same dosage as in our study.

But the cure rate was improved to 98% with higher dose of 2 tabs at 0, 24, 48 hours.

Our study showed that Artequick is as safe as Artekin but with lower ACPR in this study. Further dose finding studies will be needed to establish its efficacy in the treatment of uncomplicated falciparum malaria in adults.

## REFERENCES

1. Song Jiangpin. Efficacy and safety of oral Artemisinin-piperaquine (Artekin) compared to Artemisinin-piperaquine (Artequick) an improved fixed-dose ACT. Presentation at 2<sup>nd</sup> International Artemisinin Compounds workshop on evaluation of clinical studies, 16-17 Jan 2007, Gaungzhou, China.
2. Suon Seila, Song Jiangpin *et.al.* Randomized clinical trial of Artequick vs Artekin and Co-artem in the treatment of uncomplicated falciparum malaria in Cambodia. Presentation at 2<sup>nd</sup> International Artemisinin Compounds workshop on evaluation of clinical studies, 16-17 Jan 2007, Gaungzhou, China.
3. Trien Nguyen Trung & Dang Van Phuc. Comparison of Artequick tablets and granules in treatment of uncomplicated falciparum malaria in Vietnam. Presentation at 2<sup>nd</sup> International Artemisinin Compounds workshop on evaluation of clinical studies, 16-17 Jan 2007, Gaungzhou, China.
4. Krudsood S, Tangpukdee N, Thanchatwet V, Wilairatana P *et al.* Dose ranging studies of new Artemisinin-piperaquine fixed combinations compared to standard regimens of Artemisinin combination therapies for acute uncomplicated falciparum malaria. Presentation at 2<sup>nd</sup> International Artemisinin Compounds workshop on evaluation of clinical studies, 16-17 Jan 2007, Gaungzhou, China.
5. Song Jianping, Wang Wenlong, Li Haibo, Ou Fengzhen & Li Guoqiao. The comparison of parasitocidal speed with different doses of Artemisinin in clinical study. Presentation at 2<sup>nd</sup> International Artemisinin Compounds workshop on evaluation of clinical studies, 16-17 Jan 2007, Gaungzhou, China.
6. Soruchai K, Tangpukdee N, Wilairatana & Looaresuwan S. Clinical trials of Artequick in Thailand. Presentation at 2<sup>nd</sup> International Artemisinin Compounds workshop on evaluation of clinical studies, 16-17 Jan 2007. Gaungzhou, China.

## Hepatitis B surface antigen sero-prevalence in two border towns near Thailand

*\*Khin May Oo, \*Yi Yi Kyaw, \*Ohmar Lwin, \*\*Aye Aye Yee, \*\*\*San San Oo,  
\*\*\*\*Khin Win, \*\*\*\*\*Myo Khin & \*\*\*\*Khin Pyone Kyi*

\*Experimental Medicine Research Division

\*\*Nuclear Medicine Research Division

\*\*\*Blood Programming Research Division

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

\*\*\*\*\*Department of Medical Research (Central Myanmar)

We investigated the prevalence and associated factors of hepatitis B surface antigen (HBsAg) sero-positivity in a population of 380 subjects (182 males and 198 females) residing in Kawthaung Township in the southern border region and in 503 subjects residing in Tachileik Township in the eastern Shan State. Both townships are situated in the Myanmar-Thai border. Sera samples were tested for the presence of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg). HBsAg sero-positivity was detected in 7.1% (27 of 380) of subjects from Kawthaung and in 3.8% (19 of 503) subjects from Tachileik Township. In both study sites, the HBsAg sero-prevalence rate was higher among males. In Kawthaung Township, the HBsAg sero-prevalence was (8.8%) in males and 5.6% among females. Of them, 5 (18.5%) were HBeAg positive. HBsAg sero-prevalence was highest (9.5%) in the 41-60 years age group followed by 21-40 years age group. In Tachileik Township, males had a higher sero-prevalence rate (5.1%) than the females (3.2%) and the HBsAg sero-prevalence was highest (5.2%) in the 41-60 years age group followed by the 21-40 years age group. The HBeAg prevalence rate was 15.8%.

### INTRODUCTION

Hepatitis B is found throughout the world, but its prevalence varies greatly and is high in Asia, sub-Saharan Africa, the South Pacific, as well as within specific populations in South America, the Mid-East and the Arctic [1]. Worldwide, there are more than two billion people infected with HBV, out of which 350 million individuals are chronically infected with hepatitis B virus [2].

High prevalence areas include China, South-east Asia, and sub-Saharan Africa where the HBsAg carrier rate ranges from 10-20%. The prevalence rate is 3-5% in Japan, central Asia, the Middle East, Mediterranean area and Latin America and 0.1-2% in low prevalence areas such as the United States, Canada and Western Europe [3].

HBV infection is endemic in Myanmar, and studies carried out among different population groups revealed HBsAg carrier rate of 10-12% [4]. Previous community-based studies had shown that differential HBsAg prevalence rates existed in the north-eastern and north-western border regions of the country. The HBsAg prevalence is 13.2% in Muse Township situated on the Myanmar-China border in the north-east border [5] and 4.9% in Tamu Township which is situated on the Myanmar-India border in the north-west of the country [6].

HBV infections are diagnosed through the use of serological markers. The presence of HBV surface antigen (HBsAg) is the most common marker for HBV infection. Chronic hepatitis B is marked by the persistence of HBsAg in the serum of a patient for at least 6

months. Hepatitis B e antigen (HBeAg) is a marker of active viral replication [7].

In this study, the HBsAg sero-positivity and its associated factors were studied among the study populations in Kawthaung and Tachileik Townships situated in the Myanmar-Thai border region. Hepatitis B infection is endemic in Thailand, with 5 million Thais who are chronic carriers [8]. Apart from being located in the Myanmar-Thai border areas, the two townships had highly diversified ethnic populations and local customs.

## MATERIALS AND METHODS

Community-based cross-sectional studies were carried out on 380 subjects residing in Kawthaung Township situated in the southern border region and on 503 subjects residing in Tachileik Township in the Golden Triangle in the eastern border region of Myanmar. Both study places are situated on the Myanmar-Thai border. The study population included males and females within the age range of 9 months-82 years.

### *Study population*

#### (i) Kawthaung Township

The study population in Kawthaung Township was mainly made up of Bamar and Mon nationals and a combined group of other ethnic minorities. The study population included 182 males and 198 females within the age range of 7-75 years. The mean age of the males ( $35.9 \pm 11.7$  years) was older than the mean age of females ( $31.9 \pm 11.6$  years).

#### (ii) Tachileik Township

The study population was made up of Bamar, Shan, Ahkar, Larhu, Kachin and other minority groups residing in the area. The study population included 158 males and 345 females within the age ranged from 9 months to 82 years. The mean age of the males ( $27.7 \pm 19.5$  years) was significantly younger than that of females ( $35.6 \pm 19.6$  years).

### *Ethical clearance*

After obtaining informed consent from each subject and from parents and guardians of children participating in the studies, a pre-tested questionnaire was used to collect social and biological data from each subject. Clinical and family histories were carefully asked and recorded.

### *Sample collection*

Two milliliters of venous blood were collected from each subject under aseptic measures. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at  $-20^{\circ}\text{C}$  and transported back to the Department of Medical Research (Lower Myanmar) on ice packs.

### *Laboratory tests*

- (i) Sera samples from Kawthaung Township were tested for HBsAg by using the SD HBsAg one-step, rapid, immunochromatographic test from the Standard Diagnostics, Inc., Korea. Samples that were positive for HBsAg were further tested for the presence of HBeAg using the SD HBeAg immunochromatographic test from the Standard Diagnostics, Inc., Korea.
- (ii) Sera samples from Tachileik Township were tested for the presence of HBsAg by using the In-house DMR HBsAg ELISA test kit according to the Instruction Manual [9]. Sera samples that were positive for HBsAg were further tested for the presence of HBeAg using the SD HBeAg immunochromatographic test from the Standard Diagnostics, Inc., Korea.

## RESULTS

The overall HBsAg prevalence in subjects from Kawthaung was 7.1% (27 of 380) with 8.8% among the males and 5.6% among the females. HBeAg was present in 18.5% (5 of 27) of the HBsAg-positive subjects. In Tachileik Township, the overall HBsAg prevalence was 3.8% with 5.1% among

males and 3.2% in females. HBeAg was detected in 15.8% (3 of 19) of HBsAg-positive subjects. In both study sites, while HBsAg prevalence was higher among the males, HBeAg prevalence was higher among the females (Table 1).

Table 1. HBsAg and HBeAg prevalence in different genders

	No. HBsAg positive	%	HBeAg positive	%
<i>Kawthaung Township</i>				
Males	182	16	8.8	2
Females	198	11	5.6	3
Total	380	27	7.1	5
<i>Tachileik Township</i>				
Males	158	8	5.1	1
Females	345	11	3.2	2
Total	503	19	3.8	3

The HBsAg prevalence was 7.5% among the Bamars which made up more than eighty percent of the study population in Kawthaung Township, followed by 5.9% among the Mons and 4.5% among the other ethnic groups. HBsAg sero-prevalence was highest (9.5%) in the 41-60 years age group followed by the 21-40 years age group. In Tachileik Township, the highest HBsAg sero-positivity rate of 6.7% was seen in the Shan nationals which constituted more than twenty percent of the study population, followed by 2.6% among the Kachins, 2.1% among the Bamar and 1.7% among the Ahkars. HBsAg sero-prevalence was highest (5.2%) in the 41-60 years age group, followed by the 21-40 years age group (Table 2).

#### *Associated factors*

In Kawthaung Township, a significant association of HBsAg sero-prevalence was seen in subjects with history of sharing of toothbrushes (26.3% versus 6.2%,  $P=0.001$ ). HBsAg positivity was higher in subjects with tattoos (8.1% versus 6.8%,  $P=0.660$ ), liver disease (8.3% versus 7%,  $P=0.763$ ), history of undergoing surgical procedures (8.7% versus 6.3%,  $P=0.403$ ) although they were not statistically significant. HBsAg sero-prevalence had no association with ear

piercing and history of receiving blood transfusion.

Table 2. HBsAg prevalence among different age groups in Kawthaung and Tachileik Townships

Age group	No. of subjects	HBsAg positive	%
<i>Kawthaung Township</i>			
0-20 years	47	1	2.1
21-40 years	227	17	7.5
<b>41-60 years</b>	<b>95</b>	<b>9</b>	<b>9.5</b>
> 60 years	11	0	0
All age groups	380	27	7.1
<i>Tachileik Township</i>			
0-20 years	149	4	2.7
21-40 years	173	7	4.0
<b>41-60 years</b>	<b>135</b>	<b>7</b>	<b>5.2</b>
>60 years	46	1	2.2
All age groups	503	19	3.8

In Tachileik Township, HBsAg sero-prevalence was higher in subjects who had history of drug abuse (12.5% versus 3.3%,  $P= >0.05$ ), presence of tattoos (5.1% versus 3.5%,  $P=0.496$ ), sharing of toothbrushes (5.2% versus 3.7%,  $P=0.574$ ) although the difference was not statistically significant. There was also no significant association of HBsAg sero-positivity with blood transfusion, ear piercing or body piercing, or history of hospitalization.

## DISCUSSION

In Myanmar, few data existed on the HBsAg sero-prevalence in the border areas with the neighboring countries. In this study, the HBsAg prevalence and the associated factors were studied in two townships situated in the Myanmar-Thai border. Kawthaung Township is located in the southern border region and Tachileik Township is situated in the eastern border region neighboring Thailand. Except from being located on the Myanmar-Thai border, the study population was greatly diversified in the two study areas with different local customs. In Kawthaung Township, the majority of the study population consisted of Bamars while the majority of the study

population in Tachileik Township was made up of Shans and Ahkars.

The HBsAg prevalence in subjects from Kawthaung was 7.1% (27 of 380) and was higher than that of the subjects in Tachileik Township which was 3.8% (19 of 503). The HBsAg sero-prevalence rates from this study were lower than the HBsAg carrier rate of 10-12% detected in different population groups in previous studies [4].

The HBsAg sero-prevalence was higher among the males in both study places. The HBsAg prevalence was 8.8% among the males and 5.6% among the females residing in Kawthaung Township and 5.1% among the males and 3.2% in females in Tachileik Township. It was similar to the findings of a community-based study by Khin Maung Tin and co-authors where no significant difference in the prevalence for males (11.5%) and females (9.07%) was observed [10]. Lewis-Ximenex and co-workers stated that male gender also seemed to play an important role in the acquisition of HBV infection and there were approximately twice as many males as females with acute HBV infection. The gender difference in the acute cases might also reflect the increased frequency of high-risk behavior such as multiple sexual partners and drug use among men compared to women [11].

HBeAg was detected in 18.5% (5 of 27) of the HBsAg-positive subjects in Kawthaung Township and in 15.8% (3 of 19) of HBsAg-positive subjects from Tachileik Township. The HBeAg prevalence rates were comparable to the findings of Nguyen VTT and co-authors in which HBeAg was detected in 16.4% of the HBsAg-positive group in a cross-sectional sero-prevalence study carried out in Vietnam [12]. In both study sites, the HBeAg prevalence was higher among the females.

In the United States, the highest rate of hepatitis B occurs in people 20-49 years olds [13], and the findings from this study had elicited that the age-specific HBsAg sero-prevalence rate was highest in the

41-60 years age group followed by the 21-40 years age group in both study places.

In both study sites, the HBsAg sero-prevalence was lower (2.1% in Kawthaung and 2.7% in Tachileik Township) in subjects less than 20 years of age. In Tachileik Township, the highest HBsAg sero-positivity rate of 6.7% was seen in the Shan nationals, which constituted more than twenty percent of the study population. It could be associated with the local custom of applying tattoos on wrists and nape of the neck believed to ward off evil by the Shan nationals. In Kawthaung Township, the HBsAg sero-prevalence was highest (7.5%) among the Bamars.

In Kawthaung Township, a significant association of HBsAg sero-prevalence was seen in subjects with history of sharing toothbrushes (26.3% versus 6.2%,  $P=0.001$ ) which highlighted the risk associated with the sharing of personal items. HBsAg sero-prevalence was also higher in subjects with tattoos (8.1% versus 6.8%), liver disease (8.3% versus 7.0%), surgical procedures (8.7% versus 6.3%) although they were not statistically significant. HBsAg prevalence had no association with ear piercing and history of receiving blood transfusion.

In Tachileik Township, HBsAg sero-prevalence was higher in subjects who had history of drug abuse (12.5% versus 3.3%), presence of tattoos (5.1% versus 3.5%), sharing of toothbrushes (5.2% versus 3.7%) although the difference was not statistically significant. There was also no significant association of HBsAg sero-positivity with blood transfusion, ear piercing or body piercing or history of hospitalization.

Findings from different studies elicited that HBsAg prevalence rates in the normal population may differ considerably within a country, depending on various local and ethnic, socio-economic, cultural, geographic, religious and other factors [14].

From this study, it is evident that the differential sero-prevalence of HBsAg and associated risk factors exist in the different



study populations residing in Myanmar-Thai border areas in the southern and eastern border regions of the country and reflects the HBsAg prevalence of 5-10% in neighboring Thailand [8].

## ACKNOWLEDGEMENT

We thank Dr. Lay Kyaw, Township Medical Officer, Kawthaung Township, Taninthayi Division and Dr. Maung Maung Yu, District Medical Officer, Tachileik Township, Eastern Shan State for their assistance during the field surveys.

## REFERENCES

1. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment and current and emerging control measures. *Journal of Viral Hepatitis* 2004; 11: 97-107.
2. Global Health Situation and Projections and Estimates, 1992. World Health Organization, Geneva, 1992.
3. Lui H. HBV, the Silent Killer among Us. [www.acma.org/journal/hepatitis\\_B\\_lui\\_01.htm](http://www.acma.org/journal/hepatitis_B_lui_01.htm).
4. Khin Pyone Kyi & Khin Maung Win. Viral Hepatitis in Myanmar. *DMR Bulletin* 1995; 9 (2):1-31.
5. Khin May Oo, Ohmar Lwin, Sandar Nyunt, Aye Aye Yee, San San Oo, Khine Win, Khin Pyone Kyi & Myo Khin. Hepatitis B surface antigen seroprevalence in a township in the north-eastern border region of Myanmar. *Myanmar Health Sciences Research Journal* 2007; 19 (3): 161-165.
6. Khin May Oo, Ohmar Lwin, Aye Aye Yee, San San Oo, Sandar Nyunt, Khine Win & Myo Khin. Differential prevalence of hepatitis B surface antigen in Northern border areas of Myanmar. Abstract of Poster No. P-0234. Presented at the 17<sup>th</sup> Conference of the Asian Pacific Association for the Study of the Liver, March 27-30, Kyoto 2007. *Hepatology International* 2007; 1:3-242 P 114.
7. Adhami T & Levinthal G. Hepatitis B-Print Version. Disease Management Project. Accessed from [www.clevelandclinicmeded.com/medical-pubs/diseasemanagement/gastro/hepatitis\\_b/hepatitis\\_b.htm](http://www.clevelandclinicmeded.com/medical-pubs/diseasemanagement/gastro/hepatitis_b/hepatitis_b.htm).
8. Pramoolsinsap C, Pukrittayakamee S & Desakorn V. Hepatitis B problem in Thailand. *South East Asian Journal of Tropical Medicine and Health* 1986; 17 (2): 219-228.
9. Instruction Manual, DMR HBsAg ELISA Test Kit. Department of Medical Research, Yangon, Myanmar
10. Khin Maung Tin, Tin Htut, Hla Myint & Tun Khin. Prevalence of hepatitis A and B in Burma. *Proceedings of the First Conference of Medical Specialities* 1981; 26-31.
11. Lewis-Ximenez LL, MR do O' Kycia, Ginuino CF, Silva JC, Schatzmayr HG, Stuver S & Yoshida CFT. Risk factors for hepatitis B virus infection in Rio de Janeiro, Brazil. *BMC Public Health* 2002; 2:26. Accessed from <http://www.biomedcentral.com/1471-458/2/26>.
12. Nguyen VTT, Mclaws M-L & Dore GJ. Highly endemic hepatitis B infection in rural Vietnam. *Journal of Gastroenterology and Hepatology* 2007; 17 645465 (P, S, G, E, B, D)
13. Viral hepatitis–Hepatitis B. Accessed from <http://www.uhs.umich.edu/health/hep.html>
14. Sobeslavsky O. Prevalence of markers of hepatitis B virus infection in various countries: a WHO Collaborative Study. *World Health Organization Bulletin* 1985; 63: 935-9.

## Utilization of basic health staff by rural community in disease management of malaria

\*Hla Soe Tint, \*Myitzu Tin Oung, \*Thein Tun & \*\*Thar Tun Kyaw

\*Department of Medical Research (Upper Myanmar)

\*\*Department of Health

This study aimed to assess the utilization of basic health staff (BHS) by rural community in disease management of malaria. A community-based cross-sectional analytic study was conducted from September 2007 to September 2008 at selected malaria endemic areas of five townships in Mandalay Division. A total of randomly selected 1500 heads of household or family members aged over eighteen years and 153 BHS were included in this study. Among 153 malaria cases, 33.3% of patients utilized BHS. Only 18.3% knew the availability of proper anti-malarial treatment with BHS. In multivariate analysis, locally contracted malaria, affordable traveling cost, reasonable treatment cost, domiciliary treatment service, shorter duration of treatment and knowledge on availability of anti-malaria treatment at BHS are significantly associated with utilization ( $p < 0.05$ ). Parallel to maintenance and strengthening BHS services including domiciliary care, information on availability of anti-malaria treatment with BHS should be given to community.

### INTRODUCTION

Malaria is the third leading cause of morbidity and first leading cause of mortality in Myanmar [1]. The malaria control program aimed for early diagnosis as well as prompt and adequate treatment in the community since September 2002. The national anti-malarial treatment policy introduced rapid diagnostic test (RDT) and artemisinin-based combination therapy (ACT) for confirmed uncomplicated *Plasmodium falciparum* malaria.

In 2004, malaria control program gave on-job training according to latest anti-malaria treatment guideline to all BHS in Upper Myanmar. RDT and ACT were made available free of charge down to sub-centers (SCs) level where midwives (MWs) are working. Township medical officer (TMO) delegates the authority of distribution to township health nurses (THN) or health assistant grade-1 (HA-1) of township health department. THN and HA-1 issue rural health center (RHC) allotments to health

assistants (HA). HA and public health supervisor grade 1 (PHS-1) manage the allocation of RDT and ACT to SCs within their jurisdiction.

Although BHS were trained and routinely supplied by program, BHS utilization by community has not been investigated in Upper Myanmar. In 2008, we conducted a cross-sectional survey in malaria endemic areas of Mandalay Division, Upper Myanmar. This study described information on BHS utilization and factors influencing utilization from both consumer and provider sides.

### MATERIALS AND METHODS

A community-based, cross-sectional analytic study was conducted from September 2007 to September 2008 at PyinOoLwin, Patheingyi, Singu, Madayar and Thabeik-kyin Townships in Mandalay Division where all BHS were trained for disease management of malaria with regular RDT and ACT supply. Using multi-staged sampling method, firstly, five townships

of malaria endemic area were selected from Mandalay Division. Next, 19 RHCs were identified as malaria endemic areas from existing list of 31 RHCs in selected townships.

Of them, 10 RHCs were randomly selected using lottery method. Then, from each RHC, five SCs were selected randomly, totaling 50 SCs. After that, from each SC, two villages (one where SC situated and the other, the most remote area furthest away from respective SC to elicit the effect of distance on utilization) were selected, totaling 100 rural villages. Finally, in each village, 15 heads of household (or family members aged over 18 years) were selected using systematic random sampling method with sampling interval of 5 achieving 1500 households. Face-to-face interview with heads of household was conducted using pre-tested, structured questionnaires. Information on socio-demographic characteristics, knowledge on malaria, healthcare seeking behavior during most recent episode of malaria attack among family members, accessibility to healthcare services, attitude and utilization of BHS were investigated through interview.

In each RHC, one focus group discussion (FGD) session with six to eight rural people of both sex and aged over 18 years was conducted at suitable SC. A total of 10 FGD sessions were conducted to get further information regarding attitude towards BHS by rural community. In-depth interviews (IDI) with 50 MWs and 10 HAs who were actually facing with problems regarding disease management of malaria were also conducted to reveal the constraints on the provider side.

#### *Data management*

Data were entered into computer using Epi-data version 3.02-software. Double data entry was done to check completeness and accuracy. Any discrepancy was corrected by reviewing the raw data. Data analysis was performed by using R-Gui statistical software.

#### *Ethical consideration*

This study was approved by the Institutional Ethical Committee of Department of Medical Research (Upper Myanmar) and written informed consent was taken from each and every participant.

## **RESULTS**

#### *Malaria prevalence*

Out of 7974 household members, 153 people reported malaria-like fever one year before survey. Among them, malaria was confirmed by RDT (61.4%) and microscopy (7.2%). Prevalence of confirmed malaria in study population was 13.2 per 1000 population.

#### *Background characteristics of malaria patients*

Median family size of households with malaria patient was five ranging from one to twelve members. Among 153 malaria patients, mean age was  $37.2 \pm 9.8$  years with a range of 18 to 64. Females accounted for 53.1% and 46.9% were males. Majority were laborers (75.3%) followed by land-owned farmers (10.0%), semi-skilled workers (8.7%), sellers (3.5%) and dependents (2.4%). Most of the patients were at middle school level (50.3%) followed by primary school level (37.7%), high school level (7.3%), illiterates (3.5%) and university or graduates (1.1%). Mean family income per month was  $42036 \pm 33257$  kyats ranging from 10,000 to 300,000 kyats.

#### *Background characteristics of BHS*

A total of 153 BHS including four THNs, one HA-1, seventeen HAs, seventeen lady health visitors, eight PHS-1s, eighty-nine MWs, and seventeen public health supervisor grade 2 (PHS-2) participated in this study. Among them, 79.1% were females and 20.9% were males. Mean age was  $36.1 \pm 10.9$  years with a range of 22 to 59 years. Of them, 45.1% were university level followed by high school (43.1%), and graduates (11.8%). Mean duration of service

was 13.5±10.5 years ranging from 1 to 35 years. Approximately half (49.7%) of BHS received training on the latest anti-malarial treatment guideline for 2 times, 22.9% for 1 time, 27.4% for 3 or 4 times.

#### *Accessibility to BHS*

Most of families (64.5%) reside within 1.5 kilometers (km) away from RHC or SC with a range of 0.2 km to 7.5 km. Among people who reported malaria-like fever within one year (n=153), BHS treated 3.3% of patients. Of them, 41% received domiciliary care by BHS. The rest came to RHC or SC by motorcycle (22%), by cart (16%), on bicycle (14%) and on foot (7%). The average traveling cost was 476±293 kyats which ranged from 200 to 1000 kyats. Approximately half (52.9%) of patients could afford traveling cost. Although diagnosis by RDT and treatment with ACT were given free of charge, they had to pay for other ancillary treatments like vitamins, anti-pyretics and injection glucose etc. The average treatment cost for each visit was 1098±387 kyats with a range of 500 to 1500 kyats. Concerning with treatment cost, 61% stated 'it is expensive' but remaining 39% stated 'it is reasonable'. The mean duration of treatment was 2.9±1.1 days. The majority of respondents (82.7%) knew the name of BHS assigned in their locality. However, only 18.3% of respondents knew that proper antimalarial treatment could get from BHS with free-of-charge or at a reasonable price.

#### *Accessibility to other non-BHS healthcare providers*

The non-BHS healthcare providers from whom malaria patients sought treatment were general practitioners (GP) (46.4%), clinics opened by non-governmental organizations (NGOs) (6.5%) and township hospitals (3.3%). Mean distance between home and non-BHS healthcare provider was 2.7±1.9 km ranged from 1.0 km to 8.0 km. Only 3.9% received domiciliary care. The transportation included cart (53.9%), motorized vehicles (23.5%), bicycle (9.8%) and foot (8.8%). The average traveling cost was 1622±677 kyats which ranged from

1000 to 4000 kyats. The majority of non-BHS users (83.3%) can afford traveling cost. The treatment cost was 5873±1397 kyats with a range of 1500 to 10,000 kyats. Most of the non-BHS users (77.5%) can afford the treatment cost. The mean duration of treatment was 3.8±0.7 days.

#### *Healthcare seeking behavior regarding malaria among rural people*

Among 153 reported malaria cases, the sources of initial treatment were self-treatment (34%), malaria drugs from drug shops (32%), visit to BHS (16.3%), traditional practitioners (10.5%), GPs (4.6%) and NGO clinics (2.6%). The initial treatment cured 20.9% of the cases. The uncured cases (n=121) sought for second providers. The second providers included GPs (37.2%), BHS (20.7%) and others (42.1%). Approximately half (47.1%) were cured with second treatment. The third treatment included GPs (65.3%) and BHS (10.2%). In total, 16.3% were admitted to hospital.

#### *Attitude of rural people on BHS with regards to malaria treatment*

Only 18% of rural people accepted that BHS could treat malaria properly and 68.6% did not regard BHS as health care provider for malaria. The majority (82.7%) knew the name of BHS and 81.4% stated that BHS had good social dealing.

Qualitative findings revealed that rural people appreciated good social dealing and domiciliary care.

*"Midwife is very kind-hearted. She is ever-smiling. She visited our house whenever we call her (whenever someone in our family get ill)".*

A villager in Thabeikkyin

#### *The obstacles and constraints of BHS in disease management of malaria*

IDI with BHS revealed the shortage of RDT especially in malaria season. The median allotment of RDT for each MW was five ranging from two to fifteen and that of ACT was two strips ranging from one to fifteen despite the average number of clinically

Table 1. Factors influencing utilization of BHS by rural community

Factor	Total no.	Utilization of BHS		Crude odds ratio (95%CI)	Adjusted odds ratio (95%CI)
		No (%)	Yes (%)		
<b>Age (years)</b>					
Old (>40)	58	37 (63.8)	21 (36.2)	1	-
Young (≤ 40)	95	65 (68.4)	30 (31.6)	0.8 (0.4-1.6)	-
				p=0.556	
<b>Gender</b>					
Male	70	46 (65.7)	24 (34.3)	1	-
Female	83	56 (67.5)	27 (32.5)	0.9 (0.5-1.8)	-
				p=0.818	
<b>Education</b>					
≥uni-versity	67	45 (67.2)	22 (32.8)	1	-
≤high school	86	57 (66.3)	29 (33.7)	1.0 (0.5-2.1)	-
				p=0.908	
<b>Marital status</b>					
Single	38	29 (76.3)	9 (23.7)	1	-
Married	115	73 (63.5)	42 (36.5)	1.9 (0.8- 4.3)	-
				p=0.146	
<b>Household size</b>					
≤5	86	54 (62.8)	32 (37.2)	1	-
>5	67	48 (71.6)	19 (28.4)	0.7 (0.4-1.3)	-
				p=0.249	
<b>Family income (Kyats)</b>					
>50000	30	20 (66.7)	10 (33.3)	1	-
≤50000	123	82 (66.7)	41 (33.3)	1.0 (0.4- 2.3)	-
				p=1.000	
<b>Type of malaria</b>					
Imported	97	73 (75.3)	24 (24.7)	1	-
Local	56	29 (51.8)	27 (48.2)	2.8 (1.4-5.7)	3.6 (1.1-11.7)
				p=0.003	
<b>Treatment available at BHS</b>					
Not know	92	77 (83.7)	15 (16.3)	1	-
Know	61	25 (41)	36 (59)	7.4 (3.5-5.7)	9.7 (3.1-30)
				p=0.000	
<b>Place of service</b>					
At facility	132	98 (74.2)	34 (25.8)	1	-
Domiciliary	21	4 (19)	17 (81)	12.3(3.9-8.9)	28.1 (3.8 - 29.7)
				p=0.000	
<b>Treatment duration (Days)</b>					
>3	78	67 (85.9)	11 (14.1)	1	-
≤3	75	35 (46.7)	40 (53.3)	7 (3.2 -15.2)	14.9 (4.2- 52.5)
				p=0.000	
<b>Traveling cost</b>					
Can afford	112	85 (75.9)	27 (24.1)	1	-
Can't afford	41	17 (41.5)	24 (58.5)	4.4 (2.1-9.5)	14.1 (3.8- 52.6)
				p=0.000	
<b>Treatment cost</b>					
Can afford	110	79 (71.8)	31 (28.2)	1	-
Can't afford	43	23 (53.5)	20 (46.5)	2.2 (1.1-4.6)	3.9 (1.3-12.6)
				p=0.031	

suspected malaria cases per month was around 15. The RDT being distributed by malaria program was only for *Plasmodium falciparum*. BHS preferred multi-species RDT for better diagnosis and they would like to do microscopic diagnosis if program supplies stains, slides and some refresher course on malaria microscopy.

#### Factors influencing utilization of BHS

Table 1 shows factors influencing utilization of BHS by the community. There was no significant difference in background characteristics such as age, sex, education, marital status, household size and family income for choosing type of health service provider. Good knowledge on symptoms and complications of malaria had no significant effect on choice of provider. The patients were more likely to seek care from BHS if they knew malaria season (OR=2.7, 95% CI 1.1 to 6.4). The odds of being a BHS user was higher for closer distance between the patient's residence and the public health facility (OR=2.1, 95% CI 1.1 to 4.3), good social-dealing of the assigned BHS (OR=3.6, 95% CI 1.2 to 11.1) locally contracted malaria, aware-ness on availability of anti-malaria treatment with BHS, acceptance of BHS as malaria treatment provider (OR=7.2, 95% CI 3.3 to 15.7) and domiciliary treatment service, affordable traveling cost and reasonable treatment cost.

In the binary logistic model, the use of BHS service was associated with awareness on availability of anti-malaria treatment with BHS, domiciliary treatment service, shorter duration of treatment, locally contracted malaria, affordable traveling cost and reasonable treatment cost.

## DISCUSSION

The prevalence of confirmed malaria in our study was 13.2 per 1000 population which is slightly higher than nationwide malaria morbidity rate in Myanmar i.e. 10 per 1000 population [1] reflecting the endemicity of our study area.

One-third of malaria patients utilized BHS which was higher than previous studies such as 12.8% at Mudon Township in 1993 and 10.4% in Bago Division, Mandalay Division, and Mon State in 1999 [2, 3]. However, the utilization rate was still low. Globally, the use of official sector by malaria patients ranged from 10 to 99% worldwide [4].

In this study, there was no significant difference in background characteristics such as age, sex, education, marital status, household size and family income for provider choice. A study on malaria treatment in Brazil reported that gender and age were unimportant in choosing between public and private healthcare [5]. Regarding factors influencing utilization of BHS, locally contracted malaria cases, shorter duration of treatment, affordable traveling cost and reasonable treatment cost are major factors. Utilization of BHS due to cheaper traveling as well as treatment cost was indicated in a study of Africa [6]. Therefore, BHS utilization should be improved by giving more attention on imported malaria cases (malaria patients acquired from outside), effort on early diagnosis, searching for new anti-malarial drugs with shorter treatment courses and effort on distribution by free-of-charge or franchise.

In our results, vicinity to health facility was associated with increased utilization. The long distance to health facility is likely to be a barrier to utilization of BHS because of traveling cost and wasting time. In the meantime, domiciliary care practiced by BHS was found to be important factor for improving utilization of BHS. It was supported by a study in Brazil [5]. Therefore, BHS utilization should be promoted through practicing domiciliary care.

The odd of being a BHS user was higher if respondents knew that proper anti-malarial

treatment could get from BHS with free-of-charge or at a reasonable price. However, only 18.3% of respondents knew the availability of proper anti-malarial treatment with BHS. Moreover, most of the respondents initially started self-treatment with home-made remedies or purchased drugs from drug shops. Therefore, parallel to maintenance and strengthening BHS services including domiciliary care, information on availability of anti-malaria treatment at BHS should also be disseminated to community.

### ACKNOWLEDGEMENT

We thank all BHS and malaria patients participated in this study for their keen participation. We would like to express our gratitude to World Health Organization, Geneva, Switzerland, for funding support to conduct this study.

### REFERENCES

1. Ministry of Health. Health in Myanmar. Nay Pyi Taw, Myanmar. 2008: pp. 57.
2. Thein Tun, Soe Aung, Soe Myat Tun, Myint Myint Soe, San Shwe & Maung Maung Toe. Role of Volunteer Health Workers in Malaria Control in Mudon Township, *Paper presented at Medical Research Congress of Department of Medical Research, Ministry of Health, Yangon, Myanmar, 1993.*
3. Ejov MN, Thein Tun, Soe Aung, Saw Lwin & Khin Sein. Hospital-based study of severe malaria and associated deaths in Myanmar. *Bulletin of the World Health Organization* 1999; 77: 310-314.
4. McCombie SC. Treatment seeking for malaria: a review of recent research. *Social Science Medicine* 1996; 43: 933-945.
5. De Bartolome CAM & Stephen AV. Choosing between public and private health-care: A case study of malaria treatment in Brazil. *Journal of Health Economics* 1995;14:191-205.
6. Gilson L. The lessons of user fee experience in Africa. *Health Policy and Planning* 1997; 12: 273-85.

**Lead contamination of common Myanmar foodstuffs prepared/ stored in ceramic glazed potteries**

*\*Phyu Phyu Aung, \*Aye Aye Than, \*Theingi Thwin, \*\*Khine Khine Lwin, \*Thidar Khine, \*\*Khin Taryar Myint & \*\*\*Khin Myat Tun*

\*Nutrition Research Division

\*\*Pharmacology Research Division

\*\*\*Department of Medical Research, Lower Myanmar

Possible contamination of lead in common Myanmar foodstuffs prepared/ stored in glazed potteries (GP) was studied by measuring the lead concentration and pH of them before and after the storage/preparation in glazed potteries. The findings were compared with those which were prepared/stored in lead free plastic containers. Before the storage, all the food samples except Ngan-pyar-ye (fish sauce) were not found to have detectable lead content by atomic absorption spectrophotometer. After the storage/preparation in GP their lead levels ranged from 0.5 to 17.1 mg/kg (wet weight) with the pH range of 3 to 6. Lead levels of the control samples were non-detectable, except Ngan-pyar-ye which was previously contaminated. Mean lead contents of 4% acetic acid stored in GP were 23.1, 29.0, and 30.8 mg/kg (ppm) on day 1, 2, and 3, respectively.

## INTRODUCTION

The fact that lead-glazed pottery could result in poisoning was known in antiquity and fermented liquors stored in these containers as a source of lead poisoning have a long story [1]. In the second and first century BC wine was stored in the earthenware and drinking of it by aristocratic women have resulted in chronic lead poisoning and sterility. It may have well contributed to the rapid extinction of the aristocratic class. From this reasoning lead poisoning has been alleged to have contributed to the fall of the Roman Empire [2, 3].

Various authors in 1919 investigated earthenware containers and showed that when they were used for storing or cooking food containing malic or citric acids, these plumbo-solvent acids could lead to serious contamination of foodstuffs with lead [4]. The contribution of food to man's exposure to lead has been under study for many years beginning with the study of Kehoe *et al.*

[5, 6, 7] in 1933, who found lead in every item of food in both industrial and primitive societies. The fact that the amount of lead leached from glazed and enamelled foodware in contact with foodstuffs are dependent on inter alia the composition of the glaze/enamel, the firing conditions, pH of the food and lead-glazed utensils has been noted [8, 9, 10]. The United States Potters' Association and the United States Food and Drug Administration have defined 7 ppm as the maximum lead release of glazes recommended for use on ceramic items [11].

Knowing the importance of lead exposure from foodstuffs "Joint FAO/WHO Expert Committee on Food Additives (JECFA)" recommended in 1972 that for adults, the intake of lead via the diet should not exceed 3 mg/week which is equivalent to about 50 µg/kg body weight per week [4, 11].

In Myanmar, many foodstuffs like Hmyingapi (fish paste), Ngapi-ye-cho (fermented fish), fermented vegetables etc. are

prepared/ stored in ceramic glazed pottery. These foodstuffs are widely eaten by our people and no study has ever been done on the lead levels of these foodstuffs. The main purpose of the present study was to determine the lead contamination of common foodstuffs in order to highlight the deleterious effect of storing/preparing foods in ceramic glazed pottery.

## MATERIALS AND METHODS

Hmyin-ngapi (fish paste), Ngapi-ye-cho (fermented fish) and Ngan-pyar-ye (fish sauce) were bought from the Government shops, where they were not stored in glazed pottery. Equal amount of each item were stored in glazed pottery (GP) and lead free plastic containers (LFPC). Their pH and lead levels were measured (in duplicate samples) before and 2 and 4 weeks after the storage.

Fresh vegetables: Monnyin (mustard green), Pepin-pauk (sprouted beans), Ga-zun-ywet (water green) and fresh fruits: Da-nyin-thee (apes earing) and Shauk-thee (lemon) were bought from the bazaar for fermentation. Fermented rice water was prepared first and equal amounts were placed in the different containers. About 50 gm each item of vegetables were then put into each type of container separately. Lead levels of fresh rice water and common salt (used in fermentation) were measured before the fermentation. Only 1.8 mg of salt was added to 100 gm of food. One litre of rice water was placed in each container. pH and lead levels of the food items in each container (in duplicate samples) were measured before and 2 and 4 days after the storage (when they were in the condition to be consumed).

Kyan-ma-saing (fermented mustard green) was prepared and stored the same as above but the pH and lead levels were measured before and 5 and 7 days after the storage (when they were in the condition to be consumed). Salted apes earing and lemon were also prepared as above except that fermented water was not included. The

same amount of salt was used for the preparation. pH and lead levels (in duplicate samples) were measured before and 2 and 4 weeks after the storage (when they were in the condition to be consumed). The aforementioned common Myanmar foodstuffs that were already prepared/ stored in glazed pottery were also bought from the randomly selected 4 bazaars. Again the samples were bought at random in a way a consumer might choose such products. Their wet weight, pH, and lead levels were measured. Four per cent acetic acid solution was filled to the top of the different containers as a testing procedure for lead leaching [1]. For each type of container, a number of three were treated with 4% acetic acid and stored for 3 days. Lead levels and pH values were determined each day.

### *Determination of lead level by the atomic absorption spectrophotometer (AAS)*

#### Solid foodstuffs

Pre-weighted wet samples were dried in the oven. Dry weights of the samples were measured also. They were ashed in a temperature-programmed furnace. The temperature was gradually increased from ambient to 450°C. The ash was moistened with water and the water was then evaporated on a hotplate prior to re-ashing. This procedure was repeated until the wet ash was completely free from visible carbon particle. The ash was then dissolved in 5 ml of concentrated hydrochloric acid. The solution was evaporated to dryness and the residue was finally dissolved in 100 ml of distilled water.

Lead levels were then analysed by AAS (Pye UNicam SP 9) with background correction. It was determined at 217 nm with an oxidising air-acetylene flame. The precision and accuracy were checked by analysing 3 standard solutions. The sensitivity of the AAS was 0.1 ppm.

#### Liquid foodstuffs

The samples were filtered before being



introduced into the AAS. Lead levels were analysed as above.

#### pH measurement

Pre-treated fluid samples were measured by pH meter and the solid samples were measured by using the pH paper.

### RESULTS

Table 1 shows the lead levels and pH values of common Myanmar foodstuffs before and after storage/preparation in GP or LFPC. Before storage, lead levels of all the food items under study were non-detectable (<0.1 mg/kg wet weight) except fish sauce which had 3.03 mg/kg (3.03 ppm). The pH values of fish paste, fermented fish and fish sauce were 6 and those of other food items ranged from 3 to 4. Seven food items were selected for preparation/storage, which were supposed to be the most commonly consumed. Those stored in LFPC were found to have non-detectable lead levels after a month's storage (4 weeks) with the exception of fish sauce, which was contaminated with lead prior to storage. But its lead level did not rise with storage in LFPC.

Table 1. Lead levels of common Myanmar foodstuffs before and after storage or preparation in glazed pottery and plastic containers

Food item	pH	Lead content (mg/kg)					
		Before storage	After storage				
			Glazed pottery (GP)		Plastic container (LFPC)		
			2 weeks	4 weeks	2 weeks	4 weeks	
Hmyin-ngapi (Fish paste)	6	ND	4.5*	5.9*	ND	ND	
Ngapi-ye-cho (Fermented fish)	6	ND	4.2*	5.2*	ND	ND	
Ngan-pyar-ye (Fish sauce)	6	3.03	4.1*	4.7*	3.03	3.03	
Da-nyin-thee (Apes earring)	4	ND	0.5	1.2	ND	ND	
Shauk-thee (Lemon)	3	ND	10.4*	17.1*	ND	ND	
Fermented vegetables	3.3	ND	5.2* (2days)	6.3* (4days)	ND	ND	
Monnyin (Mustard green)	4	ND	4.9* (3days)	5.8* (7days)	ND	ND	

ND = Non-detectable

\* = More than permissible level

Those samples stored in GP contained more than maximum permissible level (2 mg/kg, Codex Alimentarius) except for salted Da-nyin-thee (0.5 mg/kg). Lead level of fish sauce increased from its pre-storage level. It was also found that lead levels increased with longer duration of storage. Salted lemon, which had lowest pH of 3.0 had highest lead level.

In Table 2, lead levels in similar food items obtained from randomly selected 4 bazaars in Yangon are presented. Duration of storage for fish paste, fermented fish, and fish sauce was informed to be whole year round. With the exception of fish sauce, lead levels of the rest two items were higher than our laboratory samples stored in GP. The lead levels in salted and fermented foodstuffs as above, purchased from the randomly selected bazaars are also shown in Table 2. Their pH values were similar to those of our laboratory samples. They were

Table 2. Lead level in common Myanmar foodstuffs from bazaars

Food Item	pH	Lead content (mg/kg)
	Mean ± SD (range)	Mean ± SD (range)
Hmyin-nga-pi	5.3 ± 0.5 (5-6)	6.9 ± 0.6 (6.3-7.6)*
Ngapi-ye-cho	4.8 ± 0.5 (4-5)	6.9 ± 0.3 (6.5-7.2)*
Ngan-pyar-ye (Fish sauce)	5.8 ± 0.5 (5-6)	3.9 ± 0.3 (3.4-4.1)*
Salted Da-nyin-thee	4.1 ± 0.03 (4.0-4.1)	1.1 ± 0.5 (0.5-1.7)
Fermented vegetables	4.0 ± 0.10 (3.9-4.1)	1.0 ± 0.4 (0.6-2.1)
Monnyin (Mustard green)	4.1 ± 0.10 (4.0-4.3)	1.4 ± 0.7 (0.6-2.1)

\* = More than maximum permissible level

also contaminated with lead although the lead levels ranged within the permissible limit (i.e. highest limit irrespective of the nature of the food (2 mg/kg) except one fermented mustard green sample from Hlaing Township bazaar. The results of lead leaching tests for different containers are presented in Table 3. From the GP large amount of lead was leached (well above the maximum permissible concentration of 7 ppm). Even in the GPs the amount of lead leached varied widely. Leaching increased with longer contact with acetic acid.

Table 3. Lead levels in 4% acetic acid solution after storage in glazed pottery/ plastic containers

Type of container	Duration of storage (hrs)	pH	Lead content (ppm)
<i>Glazed pottery</i>			
No.1	24	2.6	42.6*
	48	2.6	54.4*
	72	2.6	55.8*
No.2	24	2.6	8.7*
	48	2.6	12.5*
	72	2.6	14.6*
No.3	24	2.6	17.7*
	48	2.6	20.2*
	72	2.6	22.0*
<i>Plastic</i>			
No.1	24	2.6	ND
	48	2.6	ND
	72	2.6	ND
No.2	24	2.7	ND
	48	2.7	ND
	72	2.7	ND
No.3	24	2.6	ND
	48	2.6	ND
	72	2.6	ND

ND = non-detectable

\* = more than maximum permissible level (7 ppm)

Table 4. Maximum permissible lead levels of foods (Codex Alimentarius, 1999)

Maximum lead level (mg/ kg)	Food
0.1	<i>Edible oil</i> : soyabean oil, sunflower seed oil, sesame seed oil, etc. lards, rendered pork fat, margarine
0.1 TE	<i>Edible oil</i> : coconut oil, palm oil, palm kernel oil, etc.
0.3 UR	<i>Juices</i> : orange, apple, tomato, grape, grapefruit, etc.
0.3	Conc. pineapple juice with preservative
0.5	Cocoa butters
0.5 TE	Canned bouillon and consomme, fructose
1.0	Chocolate, composite and filled chocolate
1.0 UR	Lemon juice preserved exclusively by physical means
1.0 TE	White sugar, bouillon and consomme (in dry product), powdered sugar, soft sugar, glucose syrup, lactose, etc.
2.0 TE	Cocoa nibs, cocoa mass, cocoa press cake, cocoa dust, cocoa powder and dry cocoa-sugar mixtures
2.0	Edible acid casein, edible caseinates

Table 4 shows the maximum permissible levels of lead in foods adopted by the Codex Alimentarius, JECFA in 1999 [11]. The values are expressed in mg/kg wet weight. Although the nature of the food items are

different from our common Myanmar foodstuffs it can be used as a guideline. The maximum permissible levels ranged from 0.1 to 2 mg/kg depending on the nature of the food.

Lead level in rice water was non-detectable but that in common salt was found to be 1.78 mg/kg (not shown in the tables). Although common salt was used for fermentation and salting fruits, the amount added was too low (only 1.8 mg/100 gm of food) that it would not change the lead level of food to any appreciable degree.

## DISCUSSION

Lead leaching from the glazed pottery in contact with acidic food or drink have been noted in the past and actually as far back as the time of the Roman Empire [2]. Klein *et al.* in 1970 [1] reported 2 cases (one fatal) of lead poisoning in which apple juice (pH 2.6) stored in the incriminated vessel for 3 days contained 1300 mg/l. There were numerous findings of lead poisoning cases due to drinking of wine stored in earthenware vessels [1, 9, 10]. The amount of lead leached depends on the composition of the glaze, the firing conditions, pH of the food, and the temperature [8].

In our study, lead levels in Myanmar foodstuffs before storing or preparing in the glazed pottery were non-detectable (less than 0.1 mg/kg), except for fish sauce which contained 3.03 mg/kg (ppm). According to the seller, fish sauce was stored in cemented brick tanks in the factory.

Experimental study in our laboratory showed that all the food items, which were stored/prepared in GPs were contaminated with lead. Lead level of fish sauce increased after the storage. Even the food items whose pH was quite high had high lead content, more than the maximum permissible concentration of 2 mg/kg. Only the salted apes earring had lead level within the permissible range. Food item with lowest pH (pH 3), salted lemon had highest lead level, the

finding of which is in accord with those of the other studies. The more acidic the substance, the more lead would be leached [8]. Lead levels of food items with higher pH were comparable to those of fermented vegetables, which had lower pH values. It was because fish paste etc was stored for longer duration (2 to 4 weeks) than fermented vegetables (2 to 7 days only). This finding indicates that lead leaching increased with longer duration of storage.

Fermented vegetables and mustard green from the consumer's bazaars contained lead levels which were much lower than that of the similar food items of our laboratory probably because we used the new containers while in bazaars the containers were used in repetition. Duration of storage was similar in both cases. Salted apes earing on the other hand contained the comparable lead levels both from bazaar and our laboratory. It cannot be explained because the actual duration of storage from the bazaar sample was not known. Fish paste, fish sauce and fermented fish from bazaar had higher levels although they used the same container repeatedly, probably because they stored these foodstuffs for one whole year round while we stored our samples for only 4 weeks.

There is no documented evidence of acute lead poisoning by eating of acidic foods stored/prepared in glazed pottery in our country although chronic low level exposure cannot be excluded. It can be explained by the fact that although there was some amount of lead contamination, the foodstuffs were not acidic enough to cause excessive leaching of lead like those seen in wine and apple juice etc from the foreign countries whose pH values were below 3. Ours were all above pH 3 except for salted lemon, which had pH 3. But this does not refute the well-documented fact that storing or preparing acidic foodstuffs in glazed pottery is dangerous. There is still some amount of lead contamination, even exceeding the maximum permissible level

in some of the common Myanmar foodstuffs which can give rise to chronic low level lead exposure. From our study, consuming fish paste, fermented fish and fish sauce is more dangerous than eating fermented vegetables.

## ACKNOWLEDGEMENT

We would like to express our deep gratitude to the Director-General, Department of Medical Research, (LM) for allowing us to carry out this study and to the staff of Nutrition Research Division, DMR (LM) for their kind co-operation.

## REFERENCES

1. Klein M, Nawer R, Harper R & Carbin R. Earthenware containers as a source of fatal lead poisoning. *New England Journal of Medicine* 1970 (Sept); 24: 609-672.
2. Giltillan SC. Lead poisoning and the fall of Rome. *Journal of Occupational Medicine* 1965; 7: 53-60.
3. Whitehead TP & Prior AP. Lead poisoning from homemade wine. *Lancet* 1960 (Dec);17: 1343-1344.
4. Tong S, von Schirnding YE & Prapomontol T. Environmental lead exposure: a public health problem of global dimensions. *WHO Bulletin* 2000; 78 (9): 1068-1076.
5. Kehoe RA, Cholak J & Story RV. Manganese, lead, tin, aluminium, copper and silver in normal biological material. *Journal of Nutrition* 1940; 19: 579-592.
6. Harris RW & Elsea WR. Ceramic glaze as a source of lead poisoning. *Journal of American Medical Association* 1967; 202(6): 544-546.
7. WHO. Lead. *Environmental Health Criteria*. WHO, Geneva. 1974; p 7.
8. Storach S & Jorhem L. Tin and lead in foodstuffs. *Varfoda* 1982; 34 (suppl) 5: 468.
9. Heiklejohn AE. The successful prevention of lead poisoning in the glazing of earthenware in the North Staffordshire potteries. *British Journal of Internal Medicine* 1963; 20: 169-180.
10. Lane C & Lawrence AC. Homemade wine as a cause of lead poisoning: report of a case. *British Medical Journal* 1961; 2: 939.
11. FAO/WHO. Pesticide residues and contaminants. *Codex Alimentarius Abridged* 1999; 2: 239-240.

**Predictors of defaulting from anti-tuberculosis treatment  
in selected townships of Upper Myanmar**

*\*Hla Soe Tint, \*Myitzu Tin Oung & \*\*Bo Myint*

\*Department of Medical Research (Upper Myanmar)

\*\*Department of Health

Defaulting from tuberculosis (TB) treatment is one of the major barriers to its control. The cure rate and treatment completion rate can be improved if the predictors of defaulting behavior are known in advance. A case-control study was conducted in TB centers of selected townships of Upper Myanmar from August 2006 to July 2007 to investigate the predictors of defaulting from treatment. Total 400 patients (100 with defaulting behavior and 300 regular treatment takers) participated in this study. The significant predictors were age more than 25 years, migratory patients, poor patients, patients with risk behaviors, patients not received early health education, patients lived more than 5 kilometers from clinics, patients who had to travel more than 15 minutes to clinic, and bad social dealing of staff. The changeable factors like risk behavior of patients, giving adequate and timely health education and patients' satisfaction with health services should be improved to reduce the default from anti-TB treatment.

**INTRODUCTION**

Tuberculosis (TB) remains a serious public health problem worldwide although it is a curable disease. WHO estimates more than eight million new cases and 1.87 million deaths each year due to TB [1]. Myanmar is one of the 22 countries with the highest burden of TB in the world and was ranked 19<sup>th</sup> position in 2005 [2].

In Myanmar, the treatment success rate has been achieved the WHO recommended rate of 85% in 2006 [3]. Yet, defaulter prevention is still essential for sustainability of treatment success rate as well as the prevention of relapse and drug resistance.

Several studies have investigated the possible risk factors associated with default from anti-TB treatment. Studies conducted in Africa, Asia and Latin America showed different reasons for default. The main reasons are lack of time to visit a health facility regularly [4, 5], lack of money for transport [4], poverty, lack of material incentives [6] and most of all lack of infor-

mation or insufficient health education [4, 6, 7, 8].

Even though it is a well-known phenomenon, in Myanmar, there are relatively few studies on possible causes of default from anti-TB treatment. A study in Kyaington discovered that low education, lack of knowledge, ignorance and language barriers were major social factors influencing the default from treatment [9]. Social and economic problems of the patient, sense of wellbeing soon after treatment and problem encountered with health staff [10], traveling to other places, drug side effects [10, 11] were found to be the main reasons of defaulting in Yangon Division.

A better understanding of the various factors responsible for defaulting from treatment could help to achieve high cure rate and better patient compliance. Therefore, this study was conducted in Upper Myanmar to determine the predictors of default from anti-TB treatment in selected township TB centers.

## MATERIALS AND METHODS

A case-control study was conducted from August 2006 to July 2007. All patients with defaulting behavior, patients who took anti-TB treatment with missing doses for less than 2 months and patients who missed from treatment more than 2 months, were investigated as cases while the regular treatment takers who took anti-TB treatment more than 5 months duration were treated as controls. Three regular treatment takers immediately registered after patient with defaulting behavior in the township TB register were taken as controls. The factors responsible for defaulting behavior were investigated retrospectively.

This study was conducted in selected township TB centers of Kyaukse, Myitthah, Singkaing, Tadaroo, Meiktila, Myingyan and Kyaukpadaung Townships.

Almost all cases with defaulting behavior during the study period were traced and included in this study. However, minority of recent defaulters who were not accessible to follow were excluded from the study.

The sample size was calculated using the Epi-table calculator program of Epi-Info version 6.04d software in the sample size calculation tab valid for case-control studies. The input criteria for sample size estimation were: assuming odds ratio worth detecting as 2, percentage of exposure among controls as 50%, 5% alpha risk, at 95% confidence interval, 20% power of the test and a ratio of 1:3 for cases to controls. The minimum number of cases required was 98, minimum number of controls required was 294 and thus the minimum sample size was 392. A total of 400 patients, 100 patients with defaulting behavior and 300 regular treatment taking patients were involved in the study.

The township TB registers were reviewed and the sputum positive pulmonary TB patients, both new (treatment category I) and old (treatment category II) were identified. Then their treatment regularity

was noted and the patients who had defaulting behavior were identified. The characteristics of cases and controls were compared to find out the predictors of default from treatment.

The questionnaire was tested for content validity to determine whether it measured what it was intended to test. Internal consistency of the items of the questionnaire was tested calculating Cronbach's alpha. Interrater and intrarater reliability of the questionnaire were also checked.

Irregular and regular treatment takers were interviewed using a pre-tested and structured questionnaire to collect information regarding the potential predictors of defaulting such as socio-demographic characteristics, knowledge of tuberculosis, accessibility to township TB centers, receiving health education and satisfaction with healthcare providers.

Summation scores for knowledge of TB and satisfaction with healthcare service provider were rated as adequate if the total scores obtained equal or more than the mean value. Those obtained below mean value were rated as inadequate.

Data were entered into computer using Epi-data version 3.02-software in the field. The record files were checked for completeness and accuracy as soon as the completion of daily data entry. Any incompleteness in the raw data was recollected as soon as possible before leaving from the field. Data analysis was performed by using R-Gui statistical software.

### *Ethical consideration*

This study was approved by the Institutional Ethical Committee of Department of Medical Research, Upper Myanmar. The research work was inline with existing standard and routine procedures practiced by National TB Control Program. The fundamental principles of ethics and research on human participants were upheld throughout the study. For the confidentiality of the patients, interviews were carried out

after obtaining informed consent from each and every participant and the data were kept and analyzed under a secluded atmosphere.

## RESULTS

Patients with defaulting behavior were taken as cases from selected township TB centers; 22 from Kyaukse, 4 from Singkaing, 3 from Myitthah, 2 from Tadaroo, 18 from Meiktila, 28 from Myingyan and 23 from Kyaukpadaung. Three regular treatment takers immediately registered after patient with defaulting behavior from the same township TB register were taken as controls. A total of seven irregular treatment takers, three in Myingyan, another three in Kyaukse and one in Singkaing were followed up through household visits, interviewed and returned to respective TB center. Three recently defaulted patients (identified in TB register) were lost to follow up, so that excluded from the study. Another three patients with defaulting behavior were identified again to fulfill the desired sample size.

Among the socio-demographic characteristics, age, occupation, income, risk habits and HIV status revealed significant determinants of defaulting behavior in anti-TB treatment. However, there was no significant difference in education, sex, marital status and treatment category between cases and controls (Table 1).

Regarding knowledge of TB, approximately half of the patients (in both case and control groups) could not give adequate information regarding cause of tuberculosis, mode of transmission, signs and symptoms, treatment availability, time of sputum recheck and duration of treatment. No significant difference was also found regarding sources of information ( $p>0.05$ ).

Health education by township TB coordinator or assigned staff at township TB centers before and at the start of treatment revealed beneficial effect on treatment regularity (Table 2). Those patients who did not receive health education regarding mode

Table 1. Socio-demographic characteristics of patients enrolled in the study

Variable	Case n= 100 no. (%)	Control n= 300 no. (%)	Chi- square (for trend)	p value	Crude OR
<i>Age group (Years)</i>					
<25	13 (13)	96 (32)	15.55	0	1
25-44	47 (47)	134 (44.7)			2.59
45-54	14 (14)	25 (8.3)			4.14
≥55	26 (26)	45 (15)			4.27
<i>Occupation</i>					
Stable	74 (74)	257 (88)	11.13	0.001	1
Migrants	26 (26)	35 (12)			2.58
<i>Income (Kyats per month)</i>					
> 60,000	29 (29)	144 (48)	21.32	0	1
30,001-60,000	25 (25)	93 (31)			1.33
≤30,000	46 (46)	63 (21)			3.63
<i>Risk habit</i>					
Nil	33 (33)	178 (59.3)	6.59	0.01	1
Betel chewing	6 (6)	28 (9.3)			1.16
Smoking	25 (25)	59 (19.7)			2.29
Alcoholism	30 (30)	27 (9)			5.99
Multiple risk	6 (6)	8 (2.7)			4.05
<i>HIV status</i>					
Don't know	94 (94)	295 (98.3)	5.27	0.022	1
Positive	6 (6)	5 (1.7)			3.77

Table 2. Comparison on factors affecting accessibility to and satisfaction with health care provider

Variable	Case n= 100 no. (%)	Control n= 300 no. (%)	Chi- square (for trend)	p value	Crude OR
<i>Distance to clinic (km)</i>					
<5	21 (21)	168 (56.4)	33.69	0	1
15-May	24 (24)	42 (14.1)			4.57
>15	55 (55)	88 (29.5)			5
<i>Time to reach clinic (minutes)</i>					
<15	61 (61)	250 (83.6)	9.48	0.002	1
>35	39 (39)	50 (16.4)			3.2
<i>Waiting time (minutes)</i>					
≤15	41 (41)	173 (57.7)	9.83	0.002	1
16-30	43 (43)	102 (34)			1.78
>30	16 (16)	25 (8.3)			2.7
<i>Social dealing of staff</i>					
Very good	29 (29)	145 (48.3)	18.93	0	1
Good	59 (59)	148 (49.4)			1.99
Bad	12 (12)	7 (2.3)			8.57

of transmission of TB, importance of treatment regularity and danger of multi-drug resistant TB (MDR-TB) etc. were 4 times and patients who received health education during treatment were 2 times more likely to be taking treatment irregularly compared to those who received

health education before and at the start of treatment.

The distance between home and township TB center and time to reach TB center were found to be significant factors. Patients stayed 5 to 15 kilometers (km) and more than 15 km away from township TB centers were 4.6 times to 5 times more likely to be irregular treatment takers, respectively, compared to those patients stayed within 5 km from TB center. If the time to reach TB center was more than 15 minutes, those patients were more prone to be irregular treatment takers. However, cost for transportation was not a significant factor ( $p>0.05$ ).

If the waiting time was more than 15 minutes, it might push the patient to be a defaulter. If patient had to wait 16-30 minutes, the likelihood of becoming an irregular treatment taker was 1.8 times. If waiting time was more than half-an-hour, the risk of developing treatment defaulter would be as high as 2.7 times compared to those with waiting time of less than 15 minutes.

Bad social dealing of staff in township TB centers was also associated with development of defaulting behavior. The patients taking treatment at TB center assigned by staff with bad social dealing were 8.6 times more likely to be irregular treatment takers compared to those treated by staff with good social dealing. Extra-charges such as registration fees, donation etc. showed no significant effect on treatment regularity.

Fear of drug side effects, sense of wellbeing soon after treatment, initial home visit of basic health staff and type of directly observed treatment (DOT) provider were not associated with defaulting from treatment ( $p >0.05$ ).

Multiple logistic regression analysis was performed to determine the combined effect of predictors of defaulting from anti-TB treatment (Table 3). The independent variables significant at 0.05 level in bi-

variate analysis were included in multivariate model.

The significant predictors of defaulting from anti-TB treatment were: age more than 25 years (3.5 times), migratory patients (3.9 times), poor patients (monthly income 30,000 kyats or less) (2.7 times), patient with risk behaviors e.g. alcoholism, smoking, betel chewing (2.9 times), failure to give health education before and at the start of the treatment (4 times), patients stayed more than 5 km away from township TB center (4 times) and bad social dealing of staff (4.5 times).

Table 3. Multiple logistic analysis on predictors of defaulting from anti-TB treatment

Predictors	Adjusted O R	95%CI	p value
<i>Age (years)</i>			
≤25	1	Reference	
>25	3.48	(1.65-7.36)	0.001
<i>Occupation</i>			
Stable	1	Reference	
<i>HIV status</i>			
Not known	1	Reference	
Positive	3.67	(0.89-31.50)	0.057
<i>Health education</i>			
Before /start of treatment	1	Reference	
During treatment / no	4.25	(2.18-8.28)	0
<i>Distance to clinic(km)</i>			
≤5	1	Reference	
>5	4.34	(2.29-8.22)	0
<i>Traveling time to clinic (minutes)</i>			
≤15	1	Reference	
>15	2.25	(1.17-4.31)	0
<i>Waiting time (minutes)</i>			
≤15	1	Reference	
>15	1.73	(0.96-3.10)	0.065
<i>Social dealing of staff</i>			
Very good/ good	1	Reference	
Bad	4.49	(1.25-16.15)	0.022

## DISCUSSION

Default from anti-TB treatment is a serious problem in TB control. The factors associated with defaulting from anti-TB treatment found in the present study were older age group, low income patients, migratory populations, persons with risk habits such as smoking, alcoholism, betel chewing, failure to give health education, long distance between residence and

township TB centers, prolonged traveling time, prolonged waiting time, and bad social dealing of health staff. As it is a behavioral problem, risk factors of defaulting from treatment vary from one context to another. The factors associated with defaulting from treatment found in literatures which were consistent with our findings included problem encountered with health staff, traveling to other places [10], long traveling time, the quality of communication between patients and health workers [6] and deficient health education to patients [12].

On the other hand, in one study, HIV infection was described as the most important risk factor associated with treatment default [13], which is quite different from the findings of our study. This might be due to the fact that, in our study, we just categorized patients into those with HIV infection and those who could not tell their HIV status. We did not know the actual HIV status of the patients. The subjective problems of sense of wellbeing soon after treatment, fear of drug side effects, and type of DOT provider are also important risk factors for default. However, these factors were not significant for defaulting from treatment in this study.

In our study, failure to give health education was strongly associated with defaulting from treatment. Moreover, only half of the patients (in both cases and controls) had adequate knowledge about TB. Therefore, healthcare providers in township TB centers should pay more attention to health education regarding the disease, importance of treatment regularity and danger of MDR-TB before and throughout the treatment course. This study evaluated certain potential determinants of defaulting from anti-TB treatment. However, further investigations are still needed why patients do not comply with the treatment. Qualitative approaches may also be needed to investigate the subjective reasons for defaulting from anti-TB treatment.

Predictors of defaulting from anti-TB treatment were multi-factorial. Those included

changeable as well as non-changeable factors. Non-changeable factors such as relatively older age group, low income patients and migratory populations should be considered as target groups in order to conduct intervention programs. For those patients, specific measures such as social support for older age group and low income patients and a flexible approach to ambulatory treatment for migratory population could be possible solutions.

The changeable factors should be corrected if possible e.g. prohibition of risk habits and behaviors such as alcohol drinking, smoking and betel chewing. Healthcare providers should consider patients' satisfaction by reducing waiting time, and practicing good social dealing. However, those factors are hard to change and may need more resources. Therefore, giving proper health education about the disease, importance of treatment regularity and danger of MDR-TB should be considered as a vital function of township TB centers to prevent defaulting from anti-TB treatment.

## ACKNOWLEDGEMENT

We are grateful to all TB patients participated in this study for their keen participation. We would like to express our gratitude to World Health Organization, Geneva, Switzerland, for funding support to conduct this study.

## REFERENCES

1. Dye C, Scheele S, Dolin P, *et al.* Global burden of tuberculosis. Estimated incidence, prevalence, and mortality by country. *Journal of American Medical Association* 1999; 282: 677-686.
2. World Health Organization. Tuberculosis in the South-East Asia Region; The regional Report. WHO Project No: SE ICP TUB. 2008; 47.
3. National TB Control Program. Annual report. Nay Pyi Taw, Myanmar. 2007.
4. Balabanova Y, Drobniewski F, Fedorin I, *et al.* The Directly Observed Therapy Short-Course (DOTS) strategy in Samara Oblast, Russian Federation. *Respiratory Research* 2006; 7:44.



5. O'Boyl SJ, Power JJ, Ibrahim MY *et al.* Factors affecting patient compliance with anti-tuberculosis chemotherapy using the directly observed treatment, short-course strategy (DOTS). *International Journal of Tubercle Lung Diseases* 2002; 6:307-312.
6. Comolet TM, Rakotomalala R & Rajaonariora H. Factors determining compliance with tuberculosis treatment in an urban environment, Tamatave, Madagascar. *International Journal of Tubercle Lung Diseases* 1998; 2(11): 891-897.
7. Carreira Teixeira Belo I MT, Selig L, Raggio Luiz R *et al.* Choosing incentives to stimulate tuberculosis treatment compliance in a poor country in Rio de Janeiro State, Brazil. *Medical Science Monitoring* 2006; 12: 5.
8. Bam TS, Gunnebeg C, Chamroonsawasdi K *et al.* Factors affecting patient adherence to DOTS in urban Kathmandu, Nepal. *International Journal of Tubercle Lung Diseases* 2006; 10: 270-276.
9. Hla Naing & Myint Aung. Review of the social factors influencing the defaulter cases among tuberculosis patients in Kyaingtone Tuberculosis Center, Myanmar. *Myanmar Medical Journal* 1996 June; 41(2): 22-29.
10. Kyaw Khaing. Factors responsible for defaulting treatment among the new smear positive pulmonary tuberculosis, Zone 1 TB Treatment Center (1999). Yangon: Institute of Medicine (1): 2000.
11. Thuzar Han, Kyi Kyi Thinn, Win Maung & Aye Htun. Time factor and reasons related to tuberculosis treatment defaulting in Yangon (2002). *Myanmar Medical Journal* 2005; 49(1-4):19-24.
12. Morsy AM, Zaher HH, Hassan MH *et al.* Predictors of treatment failure among TB patients under DOTS strategy in Egypt. *Eastern Mediterranean Health Journal* 2003; 9(4).
13. Liam CK, Lim KH, Wong M *et al.* Attitudes and knowledge of newly diagnosed tuberculosis patients regarding the disease, and factors affecting treatment compliance. *International Journal of Tubercle Lung Diseases* 1999; 3: 300-309.

**Breast milk thiamine level of mothers with infants, clinically diagnosed as beri beri**

*\*Than Nu Shwe, \*Thein Thein Myint, \*Aye Maung Han, \*Aye Thaung, \*\*Theingi Thwin,  
\*\*Phyu Phyu Aung, \*\*Moh Moh Hlaing, \*\*Moe Thida Kyaw & \*\*Khin Myat Tun*

*\*Child Health Department, Institute of Medicine (I)*

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

The study aimed to determine the breast milk thiamine level of mothers of cases (clinically diagnosed beri beri infants), to compare with those of control mothers (infants with other diseases other than beri beri) and to investigate the factors favouring thiamine deficiency. A hospital-based, cross-sectional comparative study was carried out in Yangon Children's Hospital from February, 2001 to February, 2002. A total of 204 mothers (102 mothers of infants who were diagnosed as beri beri and 102 control mothers) were included. Questionnaire was constructed to include identification of subjects, socio-economic status, food intake and eating habits with special emphasis on thiamine intake, presenting complaints, treatment received and response to treatment. The mean breast milk thiamine level of mothers of cases ( $8.60 \pm 4.40$  µg/dl) was significantly lower than that of controls ( $10.35 \pm 6.26$  µg/dl) ( $p < 0.05$ ). Percent of mothers of cases (43.1%) who had habit of food avoidance, especially thiamine rich food were significantly higher than that of control mothers (10.8%) ( $p < 0.05$ ). Regarding cooking rice without discarding rice-boiled water, a significantly higher percentage of control mothers (10.8%) had the habit than mothers of cases (2.0%) ( $p < 0.05$ ).

## INTRODUCTION

Beri beri is a disease condition due to vitamin B1 (thiamine) deficiency. Infantile beri beri occurs commonly and typically in infants receiving milk from mothers who had thiamine deficiency. Thiamine is rich in foods like whole grain cereals, organ meats, nuts, lean cut pork etc. Myanmar people had traditionally faulty food preparation of washing rice repeatedly before boiling and throwing away of rice-boiled water, especially among illiterates and low income families. This reduces the thiamine content of rice to almost nil. Since meat/fish intakes of low income families are often low, vitamin B1 from animal source is again negligible. In lactating mothers, there is unnecessary food avoidance due to food taboos resulting in lower thiamine intake and thus the babies become high risk for vitamin B1 deficiency.

Pediatricians of our country are under the impression that beri beri is not uncommon among the infants, especially if they are breast-fed and if their mothers have thiamine deficiency. In many occasions, as the babies were very ill, a possibility of septicemia, besides beri beri, was taken into account and broad spectrum antibiotics were given simultaneously with thiamine injection. Some of the babies recovered promptly after receiving injection thiamine, although it still lacks biochemical proof for the definitive diagnosis of beri beri. As infants suffering from beri beri presenting with heart failure and/or shock is a medical emergency, providing injection thiamine could not wait for the time required to take blood samples of the infants. Thus the situation prompted to study the breast milk thiamine content of mothers as an indirect confirmatory evidence. The study thus aimed to determine the breast milk thiamine content of mothers

with clinically diagnosed beri beri infants and to compare with those of control mothers and to investigate the factors, especially on the maternal food habits favoring thiamine deficiency.

## MATERIALS AND METHODS

A hospital-based, cross-sectional comparative study was carried out in Yangon Children's Hospital from February 2001 to February 2002. Study group consisted of 102 mothers whose babies were clinically diagnosed as beri beri. One hundred and two mothers, matched in socio-economic status and infant's age, who were attending their sick infants (other than having beri beri) served as controls.

### Diagnostic criteria

Infants fulfilling the following criteria were diagnosed as beri beri.

1. Infant with severe dyspnoea/ aphonia/ cyanosis/fits
2. Hepatomegaly responding to injection B1 within 30-45 minutes

The mothers who were given vitamin B complex tablets or injection by the on-duty medical officer after admission of their infants were excluded from the study.

After taking informed consents, the selected mothers were interviewed employing the structured questionnaire. Questions included were: personal characteristics (age, parity, occupation, pattern of feeding), food habit (type of rice consumed, frequency of washing rice before cooking, way of cooking rice (whether discarding of rice-boiled water or not), habit of drinking rice water and presence of food avoidance, especially on thiamine rich food such as pork lean meat, organ meat, peas and beans during lactation). Samples of milk (20 ml) were collected from the mothers in the mornings using manual expression method and placed in the bottles containing three millilitres of acetic acid. Breast milk thiamine contents were measured with "Thiochrome method" by using flurospectrophotometer [1]. The principle of

this method depends on the oxidation of thiamine to thiochrome, which fluorescence in the ultra-violet light. Under standard conditions and in the absence of other fluorescing substances, the fluorescence is proportional to the thiochrome present, and hence to the thiamine originally present in solution.

### Statistical analysis

Statistical analysis was performed with Epi Info 6. Age, parity, occupation of mothers, and pattern of feeding were presented descriptively. Percent of cases and controls according to the type of rice consumed, habits of frequency of washing rice before cooking, discarding of rice-boiled water, drinking rice-boiled water and avoidance of thiamine rich food were compared by employing Chi square Test. Mean breast milk thiamine levels between cases and controls were compared by using Student 't' test. All statistical tests were considered to be significant at the  $p < 0.05$  level.

## RESULTS

Characteristics of mothers are presented in Table 1.

Table 1. Characteristics of cases and control mothers

Characteristics	Mothers of cases no. (%)	Control mothers no. (%)	P value
Parity			
≤3	81 (79.4)	78 (76.5)	0.61
>3	21 (20.6)	24 (23.5)	
Age			
≤ 35 years	88 (86.3)	87 (85.3)	0.84
>35 years	14 (13.7)	15 (14.7)	
Occupation			
Dependent	81 (79.4)	62 (60.8)	0.004*
Working	21 (20.6)	40 (39.2)	
Types of feeding			
Breast milk and water	83 (89.2)	83 (83.0)	0.21
Breast milk and supplementary food	10 (10.8)	17 (17.0)	

\*Significant difference between mothers with cases and control mothers

Age and parity of mothers of two groups were comparable. Regarding the occupation

of mothers, significantly more mothers with beri beri cases were dependents than controls ( $p < 0.05$ ). None of the mothers of both groups was exclusively breast fed, and majority of mothers in both categories fed their infants with breast milk and water. Only 10% of mothers of cases and 17% of control mothers had given supplementary foods of home made boiled rice or various brands of commercially available cereal powder.

Table 2. Food habits of mothers with infantile beri beri cases and those of control mothers

Food habits	Mothers of cases no. (%)	Control mothers no. (%)	P value
<i>Frequencies of rice washing</i>			
≤two times	57 (55.9)	59 (57.8)	0.78
>two times	45 (44.1)	43 (42.2)	
<i>Ways of rice cooking</i>			
Discarding rice-boiled water	100 (98.0)	91 (89.2)	0.01*
Without discarding	2 (2.0)	11 (10.8)	
<i>Habit of drinking rice-boiled water</i>			
Presence	15 (15.0)	20 (22.0)	0.21
Absence	85 (85.0)	71 (78.0)	
<i>Food avoidance habit</i>			
Absence and avoid only one type of thiamine rich food	58 (56.9)	91 (89.2)	0.001*
Presence (avoid all types of thiamine rich food)	44 (43.1)	11 (10.8)	

\*Significant difference between mothers with cases and control mothers

Table 2 shows the food habits of mothers. There was no difference in type of rice consumed and frequency of washing before rice cooking between the two groups. However, ways of rice cooking and habits of food avoidance especially with regards to vitamin B1 rich food were significantly different in mothers of two groups. Higher percent of mothers of cases discarded rice boiled water than the controls (98% vs 89%) ( $p < 0.05$ ). Significantly higher percent of mothers of cases (43.1%) had habits of food avoidance especially thiamine rich food than control mothers (10.8%) ( $p < 0.05$ ).

Table 3. Numbers of mothers of cases and control mothers by WHO cut-off point of breast milk thiamine level

Groups of breast milk thiamine level	Mothers of cases no. (%)	Control mothers no. (%)	P value
<5 µg/dl (Severely deficient)	25 (24.7)	19 (18.6)	0.12
5-9.9 µg/dl (Marginally deficient)	41 (40.2)	36 (35.3)	
≥0.01 µg/dl (Normal)	36 (35.3)	47 (46.1)	

Odd ratios: Marginally deficient = 1.49  
Severely deficient = 1.72

The mean±SE breast milk thiamine contents of mothers with beri beri cases ( $8.60 \pm 0.44$  µg/dl) were significantly lower than those of control mothers ( $10.35 \pm 0.62$  µg/dl) ( $p < 0.05$ ). According to the WHO, breast milk thiamine level is classified into three groups: severely deficient group is below 5 µg/dl, marginally deficient group is between 5 to 9.9 µg/dl and normal group is between 10 to 20 µg/dl [2]. In mothers of cases, 24.5% and 40.2% of the subjects had severely deficient and marginally deficient breast milk thiamine levels, respectively, compared to 18.6% and 35.3% in control mothers. Lower percent of mothers of cases had normal breast milk thiamine level than that of control mothers (35.3% vs 46.1%), although the differences were not statistically significant ( $p > 0.05$ ) (Table 3).

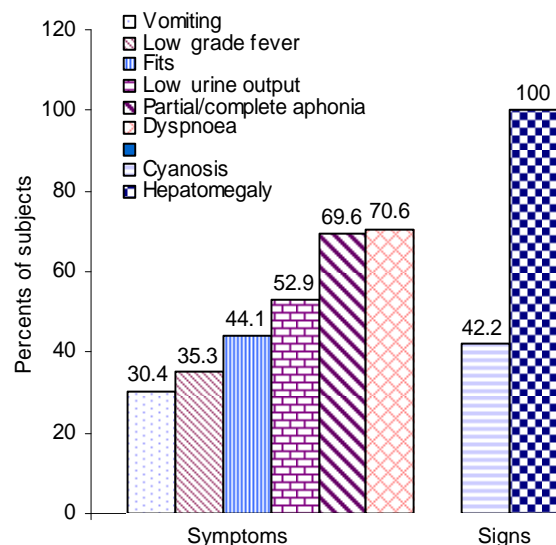


Fig 1. Clinical manifestations of beri beri cases

The age range of clinically diagnosed beri beri infants was found to be from 13 days to eight months. Majority 97.3% (99 out of 102) of the infantile beri beri cases were between the age of one and four months, The highest age frequency (49 out of 102, 48%) was found in the second month of life.

The clinical manifestations of beri beri cases are shown in Fig 1. The presenting symptoms were dyspnoea (70.6%), partial or complete aphonia (69.6%), vomiting (30.4%), low urine output (52.9%) and convulsion (44.1%). The commonest sign was hepatomegaly (100%) and the second was cyanosis (42.2%).

## DISCUSSION

Thiamine is one of the vitamin B group micronutrient, which is essential in the body. It acts as a coenzyme and is involved in energy utilizing processes of the body. It is rich in whole grain cereals, lean cut pork, leafy vegetables etc. Severe deficiency of thiamine can lead to beri beri (Singalese language - too ill to move). Beri beri is especially common in breast fed infants of mothers who are thiamine deficient [3]. In our study, higher percent of mothers with beri beri infants fed breast milk alone. By occupation also, it was found that mothers of beri beri cases were mostly dependents and did not work outside, having more time to breast feed their babies. Moreover, it can be assumed that working mothers commonly introduce supplementary food earlier than dependent mothers did. The groups (cases vs controls) in the present study were comparable in age and parity.

In our study, it was found that significantly higher percent of mothers with cases had the habit of cooking rice by discarding rice-boiled water and avoiding thiamine rich foods than those of control mothers. This can be explained by the fact that thiamine is rich in whole grain cereals especially in the outer husk of rice. Therefore, if a person cooked rice by discarding rice-boiled water, a

significant thiamine content of rice would have been lost. Moreover, a lactating mother who has a habit of avoidance of thiamine rich foods, such as, lean cut pork, pulses and beans, etc, can cause thiamine deficiency which in turn lead to low thiamine content in her breast milk.

Significantly higher percent of lower mean thiamine level of mothers with cases than that of control mothers (64.7% vs 53.9%) indicates that thiamine deficiency in lactating mothers favors the occurrence of beri beri in infants. This finding is in accord with the previous comparative study on breast milk thiamine level of mothers of beri beri cases and that of mothers of non-beri beri cases in Rangoon. They showed that there was a significant relationship between the level of thiamine in the mother's milk and occurrence of beri beri, i.e  $10.1 \pm 4.7$   $\mu\text{g}/\text{dl}$  in mothers of cases vs  $12.1 \pm 4.5$   $\mu\text{g}/\text{dl}$  in control mothers,  $p < 0.05$  [4].

Not all mothers with deficient thiamine levels lead to beri beri infants (55% of control mothers had thiamine deficiency in breast milk). This finding can be due to the fact that thiamine level of breast milk must be considerably low to occur infantile beri beri, so that it would be less than Recommended Daily Allowance (RDA), i.e., 0.17 mg/day. According to Williams (1961), mean levels of breast milk thiamine from 5-6  $\mu\text{g}/\text{dl}$  are perilous and 10  $\mu\text{g}/\text{dl}$  may be fairly safe in preventing infantile beri beri. He also reported that 0.085 mg/day could prevent infantile beri beri [5]. In this study, the mean thiamine content of breast milk of cases is 8.60  $\mu\text{g}/\text{dl}$  and those of controls is 10.35  $\mu\text{g}/\text{dl}$ . If an average breast milk output by Myanmar mothers during one to four months was taken as 855 ml, as carried out by Nutrition Research Division, Department of Medical Research Division (1976), daily thiamine intake of infants from breast milk alone would be 0.076 mg/day in infants of cases and 0.092 mg/day in infants of control mothers. Thus, 0.092 mg/day thiamine in breast milk might be not low enough to cause beri beri.

The mean breast milk thiamine levels of mothers of cases and control mothers of present study were lower than those of other findings of a comparative study where breast milk thiamine levels of mothers with infantile beri beri and control mothers were  $9.1 \pm 4.7$   $\mu\text{g}/\text{dl}$  and  $12.1 \pm 4.1$   $\mu\text{g}/\text{dl}$ , respectively [6]. In that study, the breast milk samples were taken from mothers with beri beri cases attending the Yangon Children's Hospital in 1968 but mothers from community served as controls. Higher level of thiamine levels in them might probably be due to supplementation of vitamin B complex during those days. However, in a study on thiamine nutritional status of Khayan Township (1999), the mean value of 31 lactating mothers was  $6.7 \pm 4.3$   $\mu\text{g}/\text{dl}$  [7]. The mean thiamine level of control mothers was comparable with those of mothers from Asian countries, i.e., 10.51  $\mu\text{g}/\text{dl}$  in Philippines and 10.15 in China where rice is the main staple food [8,9]. However, the thiamine level of breast milk of Myanmar mothers was significantly lower than that of the thiamine levels of American and British mothers, i.e., 14.9  $\mu\text{g}/\text{dl}$  and 14  $\mu\text{g}/\text{dl}$ , respectively [10, 11].

The age of onset of infantile beri beri is of considerable interest, as it almost exclusively occurs in the first year of life and most commonly between the first and fourth months of life [3]. This also true with our study, as the majority of the cases were between second and third month of the age. Finding of higher prevalence of beri beri in the first few months of life can be partly explained by the fact that the maternal requirement for thiamine is increased during the later months of pregnancy. Secondly, the thiamine levels of colostrum and the early lactating milk are much lower than those in the later months [3]. Finding less cases in infants of first month of life than in second or third months can be explained by the fact that the babies have sufficient reserve of thiamine in the first month of life [12].

Regarding the clinical manifestations of infantile beri beri, most of the cases had

more than one symptom or overlapping of several symptoms. The usual conditions for seeking medical treatment were dyspnoea (70.6%) and partial or complete aphonia (69.6%). Infantile beri beri may often be ushered in by an infection, even a mild one. Therefore, some of our cases (35.5%) had a low grade fever on admission and some cases were difficult to differentiate from other causes of heart failure/shock. In most of the cases, the onset of the disease was acute. The treatment given to the cases were injection thiamine only (70.6%), thiamine and antibiotics (28.4%) and thiamine and anti-convulsants treatment (1%).

From this study, it can be stated that most of the beri beri infants had mothers whose breast milk thiamine levels were lower than the cut-off point of 9.9  $\mu\text{g}/\text{dl}$ . However, not all the mothers with low thiamine levels led to beri beri infants. Therefore, the results of this study pointed out that beri beri is still existing in this country and it is a characteristic disease of rice-eating countries, particularly when polished rice is consumed. It is rarely seen in communities where rice is parboiled or undermilled. Beri beri has tended to disappear when knowledge, attitude and practice of lactating mothers will improve and they become to have various foods. Another suggestion is to give health education to the pregnant mothers to take vitamin tablets containing B1 starting from late months of pregnancy until early months of lactation.

#### ACKNOWLEDGEMENT

First, our grateful acknowledgement must go to the mothers of the sick infants for their friendly and willing help, for without their co-operation this study would have been impossible. We also thank Professor Dr. Paing Soe, Director-General, Department of Medical Research (Lower Myanmar), for kindly allowing to do this study. Our thanks also go to the Medical Superintendent of Yangon Children's Hospital, medical officers, and nurses of that hospital for their valuable help.

## REFERENCES

1. Simpson IA & Chow AY. The thiamine content of human milk in Malaya. *Journal of Tropical Pediatrics* 1956; 2(2): 69-76.
2. WHO, Geneva, 2000. The management of nutrition in major emergencies.
3. Kon SK & Mawson EH. "Human Milk", *Medical Research Council Special Report Series No. 269 (HMSO, London)*, 1950.
4. Kywe Thein, Thein Toe, Tin Tin Oo & Khin Khin Htwe. A study of infantile beri beri in Rangoon. *Union of Burma Life Science Journal* 1968; 1: 62-65.
5. Williams RR. Conquest of beri beri. Harvard University press, Cambridge, 1961; 262-283.
6. Kywe Thein, Khin Khin Tway, Tin Tin Oo & Khin Maung Naing. Studies on the thiamine level of breast milk of some Burmese mothers. *Union of Burma Life Science Journal* 1970; 3: 151-154.
7. Khin Maung Maung, Theingi Thwin, Thandar Shwe, Yin Yin Moe, Aye Aye Than & Soe Thein. A study on thiamine nutritional status of Khayan Township. *Myanmar Medical Journal* 1999; 43: 58-61.
8. Alejo G, Leon IC, Ortiliza RD, Salus EM *et al.* Studies on milk of some Filipino mothers: Normal milk thiamine levels. *The Philippine Journal of Food Science* 1965; 94 (2): 153-159.
9. Simpson IA & Chow AY. *Journal of Tropical Pediatrics* 1956; 2: 3.
10. Kon SK & Mawson EH. Wartime studies of certain vitamins and other constituents. *Medical Research Council Special Report Series No. 269*, 1950.
11. National Research Council. The composition of milks. *Bulletin of National Research Council, Washington, No. 254*, 1953.
12. Fehily L. Infantile beri beri in Hong Kong. *Caduceus* 1940; 19: 78-93.

## Characterization of *Neisseria gonorrhoeae* strains isolated from patients attending the Sexually Transmitted Diseases (STD) and gynaecology clinics in Yangon

\*Wah Wah Aung & \*\*Kyi Kyi Thinn

\*Bacteriology Research Division, Department of Medical Research (Lower Myanmar)

\*\*Department of Microbiology, University of Medicine (1)

A total of 42 *N. gonorrhoeae* strains were isolated from 200 patients (76 men and 124 women) attending STD and gynaecology clinics in Yangon during 2006 to 2008. All the strains belonged to WI serogroup. On the basis of plasmid-mediated antimicrobial resistance, they were characterized into 14 (33.3%) penicillinase producing *N. gonorrhoeae* (PPNG) strains, 6 (14.3%) tetracycline resistant *N. gonorrhoeae* (TRNG) strains, 10 (23.9%) PP/TRNG strains and 12 (28.6%) quinolone resistant *N. gonorrhoeae* (QRNG) strains. The plasmid profiles revealed as all PPNG isolates carried a 4.4 MDa penicillinase plasmid and 3 PP/TRNG isolates carried both 4.4 MDa penicillinase plasmid and 2.6 MDa cryptic plasmid. The majority of the *N. gonorrhoeae* isolates were susceptible to azithromycin, cefixime, ceftriaxone and spectinomycin. High-level ciprofloxacin resistant strains (ciprofloxacin minimum inhibitory concentration  $\geq 4$   $\mu\text{g/ml}$ ) and strains possessing multidrug resistance to first-line antibiotics, prescribed in the WHO recommended treatment regimen for gonorrhoea, were found among the tested isolates. Precise characterization of Myanmar gonococcal isolates provides benefits for the management and control of gonococcal infection and also elucidates the molecular epidemiology of gonorrhoea to some extent in Myanmar.

### INTRODUCTION

Gonorrhoea, a classical sexually-transmitted disease (STD) caused by the bacterium *Neisseria gonorrhoeae*, still remains one of the common STDs in developing countries. Approximately 62 million new cases of gonorrhoea occurred worldwide in 1999 [1]. There are some prevalence studies of gonorrhoea in Myanmar. A study carried out on 426 symptomatic women attending various clinics in Mandalay, showed that the prevalence of gonorrhoea was 2.7% in general clinics and 14.4% in STD clinics [2]. Mar Mar Nyein *et al.* reported 2.2% of gonorrhoea cases among 90 symptomatic women at the Central Women's Hospital, Yangon [3]. Thein Myint Thu *et al.* reported the prevalence of gonorrhoea was 3.8% in married women in a closed sub-urban military community [4]. The global health problem

of gonorrhoea is also concerned with the development of antimicrobial resistance in *N. gonorrhoeae*. The worldwide prevalence of gonorrhoea and the emergence of antibiotic resistant *N. gonorrhoeae* reinforce the need for surveillance of its susceptibility to antibiotics commonly used for treatment. The antibiotics included in the currently recommended WHO regimen are ciprofloxacin, ceftriaxone, cefixime, azithromycin and spectinomycin. Trimethoprim/sulphamethoxazole and kanamycin are the drugs for the alternative regimen. A large portion of gonococcal isolates worldwide are now resistant to penicillin and tetracycline [5].

Antimicrobial resistance is widespread among the strains of *N. gonorrhoeae* and occur both as chromosomally mediated resistance to a variety of agents and as plasmid-mediated resistance to penicillin (penicillinase or betalactamase producing *N. gonorrhoeae*/



PPNG) and to tetracycline (tetracycline resistant *N. gonorrhoeae*/TRNG). PPNG were first isolated in 1976 in South-east Asia and TRNG emerged there in 1980. Fluoroquinolone resistant gonococci (QRNG) appeared in several Asian countries during the early 1990s [6].

A variety of typing methods for gonococci have been used to monitor the antibiotic resistant strains, to identify the characteristics of the organisms causing the outbreaks and to recognize the geographically predominant strains. Auxotyping, serotyping, plasmid analysis and genotyping such as DNA amplification fingerprinting, ribotyping, *opa* typing, pulse field gel electrophoresis and sequencing of specific gene have been used as epidemiological tools [7].

Precise characterization of *N. gonorrhoeae* can provide valuable information on the gonococcal strain population in the community and the emergence and spread of antibiotic resistant strains. Since no vaccine exists for *N. gonorrhoeae* infection due to lack of a suitable animal model and considerable data regarding antigenic variability of the bacterium, a better knowledge of the molecular epidemiology of gonorrhoea infection will contribute to effective prevention and control measures. Thus, an in-depth study on characteristics such as serotypes, plasmid profiles and antibiotic susceptibility pattern of Myanmar gonococcal isolates will greatly benefit the management and control of this problem and also elucidate the molecular epidemiology of gonorrhoea to some extent in Myanmar.

## MATERIALS AND METHODS

### *Study design*

A cross-sectional descriptive study

### *Study population and study area*

This study was carried out on patients attending Central STD Clinic and gynaecology out-patient departments (OPDs) of Central Women's Hospital and Thingangyun Sanpya Hospital in Yangon, Myanmar.

Informed consent was obtained from eligible patients. Total study population was 200 patients comprising 76 males and 124 females, 110 were STD clinic attendees comprising 76 males and 34 females and 90 were women attending gynaecology OPDs.

### *Inclusion criteria*

- Patients presenting with urethral/vaginal discharge, urinary symptoms, inguinal lymphadenopathy and other STI symptoms
- Both sexes
- Patients aged 18 years and above
- Patients who gave informed consent

### *Exclusion criteria*

- Female patients who had menstruation at the time of specimen collection

### *Study period*

From September 2006 to August 2008

### *Specimen collection*

Demographic and clinical data of eligible patients were recorded in a proforma. Three urethral swab specimens from male patients and three endocervical swabs from female patients were collected. Urethral swab specimens were taken at least one hour after the patient had urinated. One swab was applied on two glass slides for direct smears and a second swab was inoculated into Amies transport medium for culture. The specimens were transported in an ice box to the Bacteriology Research Division, Department of Medical Research (Lower Myanmar) within three hours.

### *Direct microscopic examination*

Gram-stained smears were examined to detect gram-negative extracellular and intracellular diplococci within polymorphonuclear leucocytes.

### *Primary isolation of bacteria by culture*

The specimens collected in Amies transport medium were inoculated onto Modified Thayer Martin media and Chocolate agar and incubated at 35-36°C in a humid atmosphere containing 3-7% carbon dioxide for

24-48 hours. Colonies likely to be gonococci were identified by gram stain, oxidase test, catalase test, superoxol test and carbohydrate degradation test [8].

#### *Serotyping of isolated N. gonorrhoeae strains*

The Phadebact monoclonal GC test kit (Boule Diagnostics, Sweden) including gonococcal reagent which contains murine monoclonal antibodies to Protein IA and IB bound to non-viable staphylococci indicating WI and WII/WIII serogroups, was used to determine the serotypes.

#### *Antibiotic susceptibility testing*

Disc diffusion test and the E test (epsilometer gradient agar diffusion test) were used to determine antibiotic susceptibility and minimum inhibitory concentration (MIC) of *N. gonorrhoeae* isolates.

#### *Disc diffusion method*

Antibiotic discs (Oxoid, Hampshire, England) containing penicillin G (10 IU), sulphamethoxazole/trimethoprim (25 µg), chloramphenicol (30 µg), tetracycline (30 µg), gentamycin (10 µg), kanamycin (30 µg), amikacin (30 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), cefixime (5 µg) and spectinomycin (100 µg) were used. The results were interpreted as susceptible, intermediate and resistant according to Clinical and Laboratory Standards Institute [9].

#### *E test*

The susceptibility as well as the minimum inhibitory concentration (MIC) of penicillin, ciprofloxacin, ceftriaxone and azithromycin was determined by using E test on 42 culture-confirmed *N. gonorrhoeae* isolates. Based on MIC values described by the manufacturer (AB Biodisk, Sweden), the isolates were interpreted as susceptible, intermediate and resistant. Both disc diffusion and E test were performed simultaneously with the same inoculums. Reference strain of *N. gonorrhoeae* ATCC 49226 was used for the quality control of disc diffusion and E test.

#### *Detection of penicillinase - producing N. gonorrhoeae (PPNG) and tetracycline resistant N. gonorrhoeae (TRNG)*

The isolated colonies were tested by chromogenic cephalosporin test using nitrocefin disc, BBL, USA, to detect penicillinase-producing *N. gonorrhoeae* (PPNG). Plasmid-mediated resistance to tetracycline (TRNG) was detected by an agar diffusion susceptibility test using a disc containing 30 µg of tetracycline. TRNG produce very small zone of inhibition or none at all. The corresponding MIC of TRNG is  $\geq 16$  µg/ml.

#### *Analysis of plasmid profiles in antibiotic resistant N. gonorrhoeae isolates*

Plasmid DNA from *N. gonorrhoeae* isolates was extracted by alkaline lysis method and subjected to plasmid analysis by agarose gel electrophoresis with ethidium bromide staining. The separated plasmid DNA bands were viewed by UV transilluminator. Plasmid profile was distinguished among drug resistant isolates [10].

#### *Data analysis*

Data entry and analysis were carried out by using SPSS version 11. Univariate analysis was done to describe distribution of *N. gonorrhoeae* infection among the study population according to sex, age, occupation and education level. The culture and smear-positive cases were presented in percentages. Drug sensitivity rates of culture-positive cases were also described in percentages. Plasmid profiles of drug resistant cases were analyzed according to the pattern of drug resistance.

#### *Ethical consideration*

Research and Ethical Committee, University of Medicine (1) approved the study.

## **RESULTS**

#### *Detection of N. gonorrhoeae infection among the study population*

Of 200 specimens tested, *N. gonorrhoeae* was identified from 14 urethral swab speci-

mens from male patients and 28 endocervical swab specimens from female patients comprising a total of 42 isolates. Thus, overall detection rate of *N. gonorrhoeae* infection was found to be 21%.

#### Serotypes of isolated *N. gonorrhoeae* strains

All tested 42 *N. gonorrhoeae* isolates showed WI serogroup.

#### Antibiotic susceptibility pattern by disc diffusion method

*N. gonorrhoeae* isolates were resistant to penicillin ( $\cong 81\%$ ), tetracycline (83.3%), chloramphenicol (83.3%) and septrin (78.6%). They showed moderate resistance to injection form antibiotics such as gentamycin (52.3%), kanamycin (47.6%) and amikacin (42.8%). The isolates were sensitive to azithromycin (88.1%), cefixime (80.9%), ceftriaxone (>73%), spectinomycin (76.2%) and ciprofloxacin (>52%).

#### MIC values determined by E test

Anti-gonococcal activities of azithromycin, ciprofloxacin, ceftriaxone and penicillin by E test were expressed in range of minimum inhibitory concentrations as shown in Table 1. High-level ciprofloxacin resistance (MIC  $\geq 4 \mu\text{g/ml}$ ) was seen in 12 (28.6%) out of 42 tested isolates.

Table 1. Range of MIC values of penicillin, ciprofloxacin, ceftriaxone and azithromycin determined by E test

Antibiotic (MIC range on E test strip)	Sensitivity pattern	No. of strains	MIC values of tested antibiotic ( $\mu\text{g/ml}$ )
Azithromycin (0.016-256)	Susceptible	37	0.25-0.5
	Resistant	5	2-6
Ciprofloxacin (0.002-32)	Susceptible	22	0.032-0.064
	Intermediate	8	0.125-0.5
	Resistant	12	4-8
Ceftriaxone (0.002-32)	Susceptible	31	0.094-0.25
	Resistant	11	0.75-1.5
Penicillin (0.002-32)	Susceptible	3	0.064
	Intermediate	5	0.125-1
	Resistant	34	2-32

#### Multiple drug resistant *N. gonorrhoeae* strain

*N. gonorrhoeae* strains that were resistant to at least 2 or more antibiotics prescribed in

the current treatment regimen namely: ciprofloxacin, ceftriaxone, cefixime, spectinomycin and azithromycin, were analyzed. A total of 7 multidrug resistant *N. gonorrhoeae* strains were detected among the drug resistant isolates. Two *N. gonorrhoeae* isolates were resistant to ciprofloxacin, ceftriaxone and spectinomycin, one isolate was resistant to ciprofloxacin, azithromycin and spectinomycin and four isolates were resistant to ciprofloxacin and ceftriaxone.

Distribution of multidrug resistant strains according to the gender is shown in Table 2. Four multidrug resistant strains were found in males and 3 multidrug resistant strains were found in females. All 7 patients were STD clinic attendees and 2 women were commercial sex workers.

Table 2. Drug resistance pattern of *N. gonorrhoeae* showing multiple resistance to antibiotics prescribed in current treatment regimen

Gender	Resistant antibiotics	No. of <i>N. gonorrhoeae</i> isolates	Sub - Total
Male	Cipro+Ceftria+Spectino	1	
	Cipro+Azithro+Spectino	1	4
	Cipro+Ceftria	2	7
Female	Cipro+Ceftria+Spectino	1	
	Cipro+Ceftria	2	3

Cipro = Ciprofloxacin, Ceftria = Ceftriaxone, Azithro = Azithromycin, Spectino = Spectinomycin

#### Characterization of plasmid-mediated antibiotic resistant *N. gonorrhoeae* isolates

Characteristics of drug resistant isolates according to the following categories are shown in Table 3.

1. PPNG (beta-lactamase positive, tetracycline MIC  $\leq 16 \mu\text{g/ml}$ )
2. TRNG (beta-lactamase negative tetracycline MIC  $\geq 16 \mu\text{g/ml}$ )
3. PP/TRNG (beta-lactamase positive tetracycline MIC  $\geq 16 \mu\text{g/ml}$ )
4. QRNG (ciprofloxacin MIC of  $\geq 1 \mu\text{g/ml}$ )

Plasmid-mediated resistance to penicillin and/ or tetracycline was found in 30 (71.4%) of total 42 *N. gonorrhoeae* isolates. Twenty-

Table 3. Categories of antibiotic resistant *N. gonorrhoeae* isolates (n=42)

Categories	Criteria	No. (%) of <i>N. gonorrhoeae</i> isolates
PPNG	Beta-lactamase (+), tetracycline MIC $\leq$ 16 $\mu$ g/ml	14 (33.3%)
PP/TRNG	Beta-lactamase (+), tetracycline MIC $\geq$ 16 $\mu$ g/ml	10 (23.9%)
TRNG	Beta-lactamase (-), tetracycline MIC $<$ 16 $\mu$ g/ml	6 (14.3%)
QRNG	Ciprofloxacin MIC of $\geq$ 1 $\mu$ g/ml	12 (28.6%)

four *N. gonorrhoeae* isolates were found to be PPNG and 16 isolates were found to be TRNG. QRNG (quinolone resistant *N. gonorrhoeae* which showed ciprofloxacin MIC of  $\geq$ 1  $\mu$ g/ml) was detected by determination of MIC using E test. High-level ciprofloxacin resistance (MIC  $\geq$ 4  $\mu$ g/ml) was seen in 12 isolates.

#### Plasmid profiles among antibiotic resistant *N. gonorrhoeae* isolates

Plasmid analysis was carried out on 30 isolates exhibiting plasmid-mediated resistance to penicillin and/or tetracycline. All 14 PPNG *N. gonorrhoeae* isolates carried 4.4 MDa penicillinase plasmids and 3 PP/TRNG isolates carried both 4.4 MDa penicillinase plasmid as well as 2.6 MDa cryptic plasmid (Plate 1).

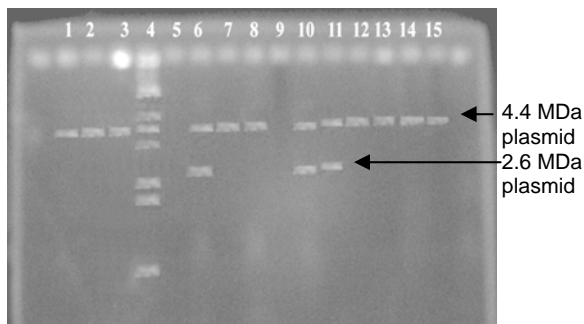


Plate 1. Plasmid analysis of *N. gonorrhoeae* isolates

Lane 1-3 - Positive samples showing 4.4 MDa plasmid

Lane 4 - Lambda HindIII DNA marker

Lane 5 - Negative control

Lane 6, - Positive samples showing 4.4 MDa 10 & 11 and 2.6 MDa plasmids

Lane 7-8 - Positive samples showing 4.4 MDa & 12-15 plasmid

## DISCUSSION

In the present study, 21% of the symptomatic patients were found to have gonococcal infection. The demographic data showed gonorrhoea patients were within the age of 24-42 years which is the reproductive age group. The people in this age group can transmit infection to sexual partners and newborns and they are also highly mobile group which can increase the spread of infection within the community.

The high infection rate was found among commercial sex workers, labourer and sedentary workers with low level of education. The similar finding was also seen in a study which stated core transmitters of gonorrhoea were of young age, low socio-economic status and high risk group for STDs eg. commercial sex workers [11].

All 42 *N. gonorrhoeae* isolates were typed as WI serogroup. WI serogroup contains protein IA, outer membrane protein. Although the present study had limitations to proceed to further serovar typing on isolated strains, the results obtained so far indicate the predominant serotype existing in our environment.

It was found that most isolates showed high resistance to oral antibiotics and moderate resistance to injection form antibiotics that were used in old STD treatment regimens. Most isolates were sensitive to antibiotics like azithromycin, ceftriaxone, cefixime and spectinomycin that are prescribed in the currently used STD treatment guidelines and  $>$ 52% of the isolates were sensitive to ciprofloxacin which is a commonly used oral antibiotic in this treatment regimen.

In a report, 6.7% of the gonococcal isolates from STD patients in Hlinethaya Township in Yangon were found to be less sensitive to penicillin, but susceptible to ciprofloxacin and ceftriaxone [12]. The present study showed decreased susceptibility of gonococcal isolates to ciprofloxacin.

In 2007, CDC no longer recommended fluoroquinolones for the treatment of gono-

coccal infection and associated pelvic inflammatory diseases [13]. Recently, there were reports on occurrence of high-level resistance to ciprofloxacin (MIC  $\geq 4$   $\mu\text{g/ml}$ ) in Argentina, Israel and Taiwan [14]. In Myanmar, although there have been no reported high-level ciprofloxacin resistant cases previously, the present study showed that the emergence of high-level ciprofloxacin resistance (MIC  $\geq 4$   $\mu\text{g/ml}$ ) which accounts for 28.6% (12/42) of tested isolates.

Although quinolones such as ciprofloxacin are recommended as the first-line of therapy for gonorrhoea in developing countries, the emergence of significant resistance to ciprofloxacin and presence of high-level resistant strains will limit the usefulness of this drug.

Emergence of multidrug resistant strains of *N. gonorrhoeae* is a serious threat in control of gonococcal infections. Wang *et al.* reported that clinical cases which showed multidrug resistance (penicillin, tetracycline and ciprofloxacin) and decreased susceptibility to cefixime occurred in Hawaii [15].

The present study was carried out cross-sectionally and all patients were out-patients, so the clinical outcome of multidrug resistant cases cannot be determined. However, all multidrug resistant patients were STD clinic attendees including 2 commercial sex workers. If they were not treated properly and effectively, the multidrug resistant strains can be transmitted to other persons and will circulate in the community. Studies of antibiotic resistant plasmids found in *N. gonorrhoeae* can predict associated antibiotic resistant genes which can provide epidemiological data for outbreak analysis and transmission of antibiotic resistant genes in the regions.

A study in Bangladesh reported 23.4% PPNG among penicillin resistant isolates and 17.5% TRNG among tetracycline resistant isolates [16]. In a study in Thailand, all penicillin resistant *N. gonorrhoeae* isolates in Bangkok Hospital during 2000-2002 produced  $\beta$  lactamase [17]. There are limited studies on plasmid-mediated gonococcal

resistance in Myanmar. Thida *et al.* reported that 45.5% PPNG, 59.1% TRNG, 36.4% PP/TRNG strains were observed among 22 *N. gonorrhoeae* strains isolated from Mandalay [18].

In the present study, isolated *N. gonorrhoeae* strains from STD were categorized into 14 (33.3%) PPNG strains, 6 (14.3%) TRNG strains, 10 (23.9%) PP/TRNG strains and 12 (28.6%) QRNG strains. The findings from the present study and previous studies in Yangon and Mandalay indicate the occurrence of plasmid-mediated resistance among gonococcal isolates in Myanmar. The 4.4 MDa plasmid is the epidemic  $\beta$  lactamase plasmid which predominated in Asia and North America. The cryptic 2.6 MDa plasmid was found in 96% of clinical isolates of *N. gonorrhoeae*. These cryptic plasmids are not associated with the virulence of gonococcal strains, pilus production, or the pilin protein and their functions are still unknown [19].

In the present study, plasmid analysis was carried out on 30 isolates exhibiting plasmid-mediated resistance to penicillin and/or tetracycline. All 14 PPNG *N. gonorrhoeae* isolates carried a 4.4 MDa penicillinase plasmid and 3 PP/TRNG isolates carried both 4.4 MDa penicillinase plasmid and 2.6 MDa cryptic plasmid. When plasmid patterns were compared to the previous study in Mandalay, the plasmid profiles of *N. gonorrhoeae* isolates from Yangon and Mandalay were found to be different as isolates from Mandalay carried 25.2 MDa tetracycline resistant plasmid and 2.6 MDa cryptic plasmid. The present information on the plasmid patterns of gonococcal isolates in Myanmar provides preliminary baseline data.

In the present study, serotyping, determination of antimicrobial susceptibility pattern and plasmid analysis methods were used to characterize the isolated *N. gonorrhoeae* strains. All the isolated strains belonged to serogroup WI and most of the antibiotic resistant strains exhibited plasmid-mediated antimicrobial resistance. The

present study also highlighted the occurrence of high-level ciprofloxacin resistant strains and multidrug resistant strains.

Further area of research on gonococcal infection including the elucidation of associated different plasmids and antibiotic resistant genes can provide epidemiological data for outbreak analysis and transmission of antibiotic resistant genes in the regions. Since no vaccine exists for *N. gonorrhoeae* infection, a better knowledge of molecular epidemiology of gonorrhoea will contribute to effective prevention and control measures.

## REFERENCES

1. World Health Organization (2001). Global prevalence and incidence of selected curable STDs overviews and estimates. Available at www.who.int./ docstore. Accessed on 12-4-06.
2. Department of Health, Union of Myanmar & Population Council, Thailand. Reproductive tract infection in Mandalay clinics, Myanmar: a cross-sectional prevalence and cost analysis study, 2002.
3. Mar Mar Nyein, Ba Thike K, Hla Hla Win, Wah Wah Aung, Mo Mo Win *et al.* Reproductive tract infection among gynaecological patients at Central Women's Hospital. *Myanmar Health Sciences Research Journal* 2000; 12(1-3): 10-13.
4. Thein Myint Thu, Wah Wah Aung, Thaw Htet, Kyaw Min Htike *et al.* Syndromic versus laboratory-based diagnosis for lower genital tract infections among married women in selected sub-urban communities, Yangon. *Myanmar Health Sciences Research Journal* 2008; 20(2):101-106.
5. World Health Organization Guidelines (2001). Guidelines for the management of sexually transmitted infections. Available at www. who. int/ mipfiles. Accessed on 12-4-06.
6. Ieven M, van Looveren M, Sudidgoadi S, Rosana, Y *et al.* Antimicrobial susceptibilities of *Neisseria gonorrhoeae* strains isolated in Java, Indonesia. *Sexually Transmitted Diseases* 2003; 30(1): 25-29.
7. Birley H, Duerden BI & Hart A. Sexually transmitted diseases: microbiology and management. *Journal of Medical Microbiology* 2002; 51: 793-807.
8. van Dyck E, Meheus AZ & Piots P. Gonorrhoea. In: *Laboratory Diagnosis of Sexually Transmitted Diseases*. WHO publication, 1999; 11-21.
9. Clinical and Laboratory Standards Institute. Performance standard for antimicrobial testing; fifteenth international supplement. *CLSI Document no. M 100-S 15*, 2002.
10. Sambrook J, Fritsch EF & Maniatis T. Molecular cloning: a laboratory manual. 2<sup>nd</sup> ed, Cold Spring Harbor Laboratory Press, New York, 1989 .
11. Jooly AM, Hammond G & Young TK. Risk factors for infection in women underlying testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Manitoba, Canada. *Sexually Transmitted Diseases* 1995; 22(5):289-295.
12. Khin Mya Lwin, San San Min, Sann Myint, Mya Thidar & Soe Nyein. Prevalence of syphilis, gonorrhoea and chlamydial infections in women attending maternal and child health care centre in Hlaing Tharya Township. *Myanmar Medical Journal* 1998; 42(3, 4): 38-42.
13. Centers for Disease Control and Prevention. Update to CDC's Sexually transmitted diseases treatment guidelines, 2006: Fluoroquinolone, no longer recommended for treatment of gonococcal infections. *MMWR* 2007; 56(14):32-336.
14. Hsueh PR, Tseng SP, Teng LJ & Ho SW. High prevalence of ciprofloxacin resistant *Neisseria gonorrhoeae* in northern Taiwan. *Clinical Infectious Diseases* 2005; 40:188-192.
15. Wang SA, Lee VMC, O'Connor N, Ziverson CJ, Ohye RG *et al.* Multidrug resistant *Neisseria gonorrhoeae* with decreased susceptibility to cefixime, Hawaii, 2001. *Clinical Infectious Diseases* 2003; 37:849-852.
16. Bhuiyan BU, Rahman M, Miah MIA, Nahar S, Islam N *et al.* Antimicrobial susceptibilities and plasmid contents of *N. gonorrhoeae* from commercial sex workers in Dhaka, Bangladesh: emergence of high-level resistance to ciprofloxacin. *Journal of Clinical Microbiology* 1999; 37:1130-1136.
17. Lawung R, Buatiang A, Jittawoutipoka T, Rittiroongrag S & Prachayasittikul V. Increasing trend of multiple resistance and genomic mobility of *Neisseria gonorrhoeae* to penicillin and quinolone. *Experimental and Clinical Sciences Journal (EXCLI, International online journal for advances in sciences)* 2005; 4: 130-140.
18. Thida. Microbiological profile of some common reproductive tract infections in Mandalay. A Thesis Submitted for the Degree of Doctor of Philosophy (Microbiology), University of Medicine, Mandalay, 2003.
19. Roberts MC. Plasmids of *Neisseria gonorrhoeae* and other *Neisseria* species. *Clinical Microbiological Review* 1989; 2 Suppl: S8-23.