

MYANMAR HEALTH SCIENCES RESEARCH JOURNAL

Volume 19, No. 3, 2007

DEPARTMENT OF MEDICAL RESEARCH

(Lower Myanmar)

No. 5, Ziwaka Road

Yangon 11191, Myanmar

CONTENTS

Syringes and needles disposal practices by House Surgeons from major hospitals in Yangon, Myanmar	115
<i>Paing Soe, Myo Khin, Kyaw Oo, Myat Phone Kyaw, S. Kyaw Hla, Tin Tin Aung, Aye Maung Han, Ne Win, Nyunt Thein, Saw Win & Than Htein Win</i>	
Stability of the recombinant hepatitis B surface antigen expression vector in genomic DNA of <i>Hansenula polymorpha</i> yeast cells (Master Cell Bank)	121
<i>Win Aung, Suk Hoon Ha, Jae Seung Kim, Yeon Hee Kim & Khin Pyone Kyi</i>	
Detection of low level viral genome (HBV DNA) by Polymerase Chain Reaction in nonresponders of plasma-derived hepatitis B vaccine produced at the Department of Medical Research (Lower Myanmar)	125
<i>Moh Moh Htun, Khin Pyone Kyi, Khin May Oo, San San Oo, Khin Ohnmar Lwin & Kenji Abe</i>	
Antimalarial activity and related chemical constituents of <i>Swertia</i> species from Kayah State	131
<i>Khin Phyu Phyu, Win Myint, Ye Htut, Mya Aye, Kyin Hla Aye & Ni Ni</i>	
Epidemiological study of snakebites cases admitted to two township hospitals of Ayeyawady Division	136
<i>Aye Aye Myint, Tun Pe & Swe Win</i>	
Detection of alpha-thalassaemia in newborn cord blood from North Okkalapa General Hospital	141
<i>Ne Win, Khine Khine Phyu, Myat Mon Oo, Mya Thandar Win, Kyaw Soe & Minn Minn Myint Thu</i>	
Family and community practices of newborn care in Pyay District	147
<i>Kyu Kyu Than, Than Tun Sein, Ko Ko Zaw, Mon Mon, Myo Myo Mon, Thein Aung, Khin Mar Aye & Myint Lwin</i>	
Contributing factors to treatment interruption in patients with tuberculosis in the selected townships with high default rates	155
<i>Htin Zaw Soe, Ye Hla, Win Maung, Thandar Lwin, Thin ThinYee & Saw Thein</i>	
Hepatitis B surface antigen sero-prevalence in a township in the north-eastern border region of Myanmar	161
<i>Khin May Oo, Ohmar Lwin, Sandar Nyunt, Aye Aye Yee, San San Oo, Khine Win, Khin Pyone Kyi & Myo Khin</i>	

Reproductive health needs of HIV positive women: client perspective 166
*Kyu Kyu Than, Poe Poe Aung, Htin Aung Saw, Tin Tin Wai, The' Maung Maung
& Nyo Aung*

Evaluation of *in vitro* antimicrobial susceptibility of *Neisseria gonorrhoeae* strains
isolated from symptomatic men and women in Yangon 173
Wah Wah Aung & Kyi Kyi Thinn

.....

Editor-in-Chief

Dr. Khin Pyone Kyi

Editor

Dr. Kyaw Min

Associate Editors

Daw Nyunt Nyunt Swe

Dr. Ni Thet Oo

Editorial Board

Prof. Dr. Aye Maung Han

Dr. Tun Pe

Prof. Dr. U Kyaw

Dr. W. Tun Lin

Prof. Dr. Ne Win

Dr. Than Tun Sein

Prof. Dr. Myo Win

Dr. Myo Khin

Prof. Dr. Kyi Kyi Thinn

Dr. Khin Myat Tun

Prof. Dr. Myo Oo

Dr. Kyaw Moe

Prof. Col. Thein Myint Thu

Dr. Thaw Zin

Asso. Prof. Dr. Soe Aung

Dr. Khin Nwe Oo

Dr. Kyaw Zin Thant

Business Manager

Dr. Ye Htut

Editorial Manager

U Aung Myo Min

Production Manager

U Ye Thway

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

Restricted for Internal Use Only

**Syringes and needles disposal practices by House Surgeons
from major hospitals in Yangon, Myanmar**

Paing Soe, **Myo Khin,*Kyaw Oo, ***Myat Phone Kyaw,****S. Kyaw Hla,
****Tin Tin Aung, ****Aye Maung Han,**** Ne Win, ****Nyunt Thein,
****Saw Win & *****Than Htein Win*

**Ministry of Health*

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

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

*****Department of Medical Science*

******Department of Health*

Needle stick injury (NSI) is regarded as an important cause of the transmission of blood-borne viruses to health care staff. There is lack of information on the occurrence of needle stick injury and needle disposal services among House Surgeons (HS) in Myanmar. With the aim of promoting measures for preventing NSIs, a hospital-based cross-sectional descriptive study was carried out to determine incidence of needle stick injury among HSs and to investigate their practice regarding injection instrument waste disposal. Two hundred and ten responding HSs at all medical wards of Yangon General Hospital, North Okkalapa General Hospital and Sanpya General Hospital, paediatric medical wards of Yangon Children Hospital and Sanpya General Hospital were investigated by self-administered questionnaire. Of them, 206 (98%) recapped the needle after giving injections, of which, 75 (36%) handled the cap during recapping process. Among 206 subjects who practiced recapping, 60 (29%) disclosed that they had experienced injury during recapping. A slightly higher rate of injury was observed among subjects who handled the cap during the recapping process as compared to those who recapped the needle without handling the cap (34% vs 26%). The majority 162 subjects (78%) separated the needle before discarding the syringe. Among those who separated needles 6.2% experienced injury during separation. The most commonly used container for discarding needle and syringes was plastic drinking water bottle (71%) followed by WHO Card Box (18%). However, only 28% of the respondents said the containers were within arm's reach and 72% of them stated that they had to walk to reach the container. Nearly 55% of all perceived that they are safe with the current practicing needle and syringe disposal system. This study showed that HSs are at risk of needle stick injury and blood-borne infections during their clinical activities while performing procedures on patients. Efforts need to be made to ensure greater awareness amongst House Surgeons about the risk of mucocutaneous and percutaneous injuries.

INTRODUCTION

In the midst of infections, disposing of waste properly is an often-overlooked but important aspect of infection prevention. The unsafe use and disposal of injection equipment continues to put patients, health-

care workers, and the general community at risk of infections such as hepatitis B or C virus and human immunodeficiency virus. Needle stick injury (NSI) is regarded as an important cause of the transmission of such blood-borne viruses to health care staff. In Taiwan where there is a high incidence of

hepatitis infections, it has been estimated that approximately 1,000 healthcare workers out of 100,000 suffer seroconversion with hepatitis B virus and hepatitis C virus through needle sticks with hollow bore needles annually [1]. Most importantly, it was reported that in countries with a high prevalence of HIV infection, one young doctor would become infected with HIV every seven or eight years [2]. The problem of NSI is considered as an important issue even in countries with low prevalence of blood borne infections. In France, it has been estimated that one surgeon in 14 might expect to be hepatitis C contaminated during his or her career, and one in 630 might be infected with HIV [3].

Among health workers at risk, medical students and interns are those with the highest risk. Nearly a third of medical students had reported sharps injury over their clinical training of which one third of those were associated with hollow bore needles [4]. In USA, based on a survey of 3,239 participants, emergency medicine residents in their first four years of training have been reported to suffer from a high rate of exposure to blood. Residents had been exposed very often, with 56% having at least one exposure, and one in 10 having four or more exposures [5]. Medical students are at risk of acquiring infections caused by NSIs, although it is unknown when NSIs are most likely to occur during medical training. Over one third (55/157) of respondents suffered at least one needle stick injury [6]. It was also reported that life-time prevalence of NSIs was 23%, ranging from 12% in first year students to 41% in fourth year students. These accidents happened most commonly during medical internships, especially during blood-taking practices; an activity that usually starts during the third year of training [7]. Among the undergraduate students in Australia, 13.8% experienced the injury during their third year training period [8].

Although several batches of medical students have completed their years as

House Surgeons at different hospitals in Myanmar, there is lack of information on the occurrence of needle stick injury and needle disposal services. The present study was conducted to explore the retrospective prevalence of needle stick injury among house surgeons and to investigate their practice regarding injection instrument waste disposal with the aim of promoting measures for preventing NSIs among the hospital medical practitioners.

MATERIALS AND METHODS

A hospital-based cross-sectional descriptive study was carried out. The frequency of injections, disposal practice of the needles and number of accidental needle stick injuries of the house surgeons at all medical wards of Yangon General Hospital, medical wards of North Okkalapa General Hospital and Sanpya General Hospital, paediatric medical wards of Yangon Children Hospital and Sanpya General Hospital were explored by self-administered questionnaire. Questionnaire included needle recapping practice, needle removal practices, and disposal practices. A total of 210 house surgeons from the four study hospitals were recruited to answer the questions. Filled questionnaires were checked by the assigned research medical officer immediately. Data recoding and entry were carried out using EpiData software. Analysis was done using SPSS version 11.5 after the data file was transferred into SPSS format. Univariate and bivariate tests were carried out to describe the occurrences and to determine differences. Differences were considered significant if $P < 0.05$.

RESULTS

Response rates

The participating house surgeons (later refer as subjects) were very cooperative and high response rates (more than 95%) were obtained for questions on needle recapping, injury obtained during recapping and

separation of needle and syringe before discarding, needle discarding practices, and disposal of containers.

Needle recapping practices and experiences of injury during recapping

Among the 210 responding subjects, 206 (98%) recapped the needle after giving injections. Of those who recapped the needle, 75 (36%) handled the cap during recapping process. The remaining 131 subjects recapped the needle without handling the cap. Among 206 subjects who practiced recapping, 60 (29%) disclosed that they experienced injury during recapping. A slightly higher rate of injury was observed among subjects who handled the cap during the recapping process as compared to those who recapped the needle without handling the cap (34% vs. 26%) (Table1).

Table 1. Occurrence of injury among subjects who recapped in different ways

How recap?	Injury during recapping		Total (%)
	Yes (%)	No (%)	
Handling the cap	25 (33.8)	49 (66.2)	74 (100)*
Without handling the cap	34 (26.0)	97 (74.0)	131 (100)
Total	59 (28.8)	146 (71.2)	205 (100)

Chi square=1.414, p=0.234*One HS did not respond.

Table 2. Method of needle separation and experience of injury among needle separators

How separate?	Injury during separating		Total (%)
	Yes (%)	No (%)	
Two hands	9 (5.9)	143 (94.1)	152 (100)
One hand	1(10.0)	9 (90.0)	10 (100)
Total	10 (6.2)	152 (93.8)	162 (100)

Needle separation practices and experiences of injury during separation

The majority 162 subjects (78%) separated the needle before discarding the syringe. The remaining 50 subjects did not separate the needle from the syringe before discarding. Of those who separated the needle from the syringe almost all 152 subjects (94%) used both hands, whereas a few 10 (6%) used only a single hand. A

small percentage (6.2%) of needle separators experienced injury during separation. Among them, 10% of those who used one hand for separation the needle from syringe had a slightly higher experience of injury during separation (10% vs 6%) than those who used the two hands method (Table 2).

Discarding practices

A variety of containers were listed. The most commonly used container for discarding needle and syringes was plastic drinking water bottle (71%) followed by WHO Card Box (18%). Other containers included storage bin (8%), tin can (2%) and plastic bags (1%) (Fig.1). Regarding the procedure for waste disposal, all participants stated that discarding the needle and syringe into the containers was unproblematic. However, only 59 (28%) of the respondents said the containers were within arm's reach and 150 (72%) of them stated that they had to walk to reach the container.

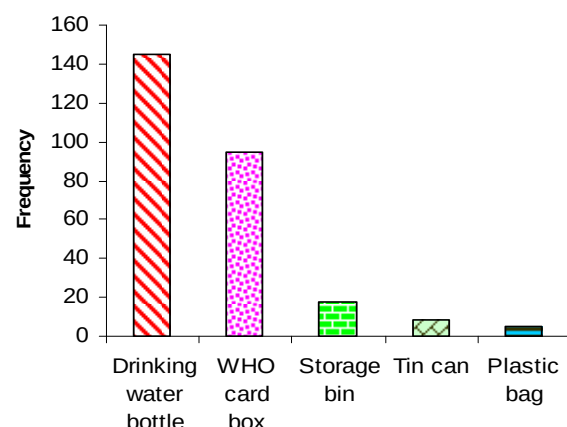


Fig 1. Types of discarding containers used by the subjects

Injury during discarding

Although it is simple to conduct, 20 % of subjects experienced that needle and/or syringes escaped from the container at the time of discarding. A very small percentage (1%) reported injury during discarding.

Practices of disposing the container

It was found that over 90% of the subjects noted that the containers were immediately emptied after being filled. It was done mainly by hospital workers (96%), although

some nurses (3%), attendants (1%) also carried out the process. Chance of injury during disposing the container was very few as only 1 % of respondents noted injury during the process.

Incidence of injury

Out of the total 211 respondents, 66 (31.3%) expressed that they had injury at least once during their HS period. The frequency of injuries was 74 amounting to 0.35 injuries per HS. Most frequent action for getting needle stick injury was during recapping (75% of all incidence injuries) (Table 3).

Table 3. Injury at various steps of disposing

Steps of disposing	N	Frequency	Percent
Injury during recapping	206	60	29.1
Injury during separating	162	10	6.2
Injury during discarding	209	2	1
Injury during emptying	209	2	1

Perceived safety on the current situation

Among the study population, nearly 55% perceived that they are safe with the current practicing needle and syringe disposal system. The remaining 45% perceived that they are less safe.

DISCUSSION

The very high response rate (more than 95%) of the subjects is very encouraging and outlines the interest of the subjects. This response rate is much higher than those obtained by investigators from United States on their medical students (77%) [4], emergency medicine residents (90%) [5]. It has been reported that only 60% of third and fourth year medical students and medical and surgical house staff replied to an anonymous questionnaire on needle stick injury [9].

The present study demonstrates that needle stick injury among the House Surgeons is not rare. At least a third of them had an

episode of NSI during their House Surgeon training period. This finding is similar to that of the risk of needle stick injury among American medical students where about a third of medical students had a sharp injury over their clinical training [4]. It has been reported that most house staff would have at least one NSI a year and NSIs most often involved disposable needles (85%), most often occurring during phlebotomy (62%), and most often when recapping a needle (54%) [10]. It has been reported that in Singapore, house officers experienced an average of 1.4 sharp and needle stick injuries per month [11].

In our study, 75% of all injuries were due to recapping the needle after injection. Both one-hand and two-hand users experienced injury in our study. The findings from our study are consistent with a study reported from Canada where 45% of needle stick injuries occurred at recapping. In that study, the results also showed between 46% and 77% of needles were being recapped and 9% to 20% of recapped needles were blood-stained [12]. Recapping devices were rarely used and two-handed recapping techniques predominated. Common reasons for recapping include inability to dispose immediately of needles properly, and sharps containers being too far away [12]. It has also been reported that recapping accounted for a higher percentage of NSI than any other activity [13]. Based on a study on medical students in India, it was found that re-sheathing or recapping the needle was responsible for causing NSI to 69% of the students [14]. Although we failed to ask the reason for recapping, more than 90% were recapping and majority of subjects (71.4%) responded that the container for discarding was placed needing to walk to reach. Only one-fourth of the subjects said the container was at within their arm's reach. This might be the reason for recapping among the subjects after practicing injection.

We failed to inquire whether the HSs reported their injury and if so any medical or psychological assistance have been

provided. They may not have reported the injury as they felt that it is not serious enough. It has also been reported that only 43% of students who were injured reported the injury, mostly because they felt it was not serious enough to constitute a serious exposure [15]. The present study focused only on NSI and did not account for other type of injuries such as those involving suture needles. In certain Institutions, injuries caused by hollow bore needles accounted for only 17% of injuries whereas surgery accounted for 70% of injuries. However, hollow bore needles could be blood filled and are usually deemed high risk [4]. Only a few incidence of injury were found during disposing and discarding container since it was not the responsibility of the house surgeons. Our study also did not take account the incidence among other staff at hospitals. .

Measures to reduce NSI have been reported by various authors. It has been reported that the introduction of a comprehensive programme to reduce NSIs led to a reduction of more than 60% over four years in a US hospital [16]. A significant, prolonged fall in needle stick injuries was demonstrated following the introduction of more, and more convenient, sharps containers at the hospital in California. More needle disposal containers were added to patient care areas and as close to the area of use as possible [15]. A similar hospital wide comprehensive multi-focused programme reduced sharps injuries by 69%. The intervention consisted of the introduction of needless systems for intravenous therapy and a new sharps disposal system. The disposal system consisted of new, wide-mouthed containers, together with a new system of changing the containers on a regular basis, and before they were full. Factors related to sharps injuries in the period before and the latest after the intervention [17]. Alternate methods for preventing needle sticks have been proposed [18]. Such measures should be outlined and adapted to local situation for use in our hospitals.

Conclusion

This study showed that House Surgeons are at risk of needle stick injury and blood - borne infections during their clinical activities while performing procedures on patients. Efforts need to be made to ensure greater awareness amongst House Surgeons about the risk of mucocutaneous and percutaneous injuries. Proper training in percutaneous procedures and how to act in case of injury should be made to reduce the number of injuries. The present study also highlights the fact that compliance with the non-recapping needle policy is poor. More education and awareness programme (lectures, videotapes, handouts); discouragement of recapping and innovative arrangements for sharps disposal should be promoted. It should be evaluated whether vaccination against hepatitis B should be offered to students before entering the clinical part of the study. It could be further stated that medical students also have a high risk for needle stick injuries, and attention should be directed to protection strategies against blood borne pathogens. Not only prevention of accidents but also post-exposure management should be frequently reiterated to the medical students at every level. Any medical practice of students should be under supervisor control.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the Medical Superintendents of Yangon General Hospital, North Okkalapa General Hospital, Yangon Children Hospital and Sanpya General Hospital for granting permission to carry out the study at their respective hospitals. The authors greatly appreciate the help provided by the Research Medical Officers.

REFERENCES

1. Shiao J, Gwo L, McLaws ML Estimation of the risk of blood pathogens to health care workers after a needlestick injury in Taiwan. *American Journal of Infection Control* 2002; 30: 15-20.

2. Karstaedt AS, Pantanowitz L. Occupational exposure of interns to blood in an area of high HIV seroprevalence. *South African Medical Journal* 2001; 91: 57-61.
3. Caillot JL, Voigloi EJ, Gilly FN. The occupational viral risk run by French surgeons: a disturbing perspective. *AIDS* 2000; 14 (13): 2061-2062.
4. Shen C, Jagger J, Pearson RD. Risk of needle stick and sharp object injuries among medical students. *American Journal of Infection Control* 1999; 27(5): 435-437.
5. CH Lee. Occupational exposures to blood among emergency medicine residents. *Academic Emergency Medicine* 1999; 6(10): 1036-1043.
6. Cervini P, Bell C. Brief report: needlestick injury and inadequate post-exposure practice in medical students. *Journal of General Internal Medicine* 2005 May; 20(5):419-21.
7. Deisenhammer S, Radon K, Nowak D, Reichert J. Needlestick injuries during medical training. *Journal of Hospital Infection* 2007 Jan; 65(1): 89-90.
8. Smith DR, Leggat PA. Needlestick and Sharps Injuries among Australian medical students. *Journal of University Occupational Environmental Health* 2005 Sep 1; 27(3): 237-42.
9. Resnic F, Noerdlinger MA. Occupational exposure among medical students and house staff at a New York City medical center. *Archives of Internal Medicine* 1995; 155: 7580.
10. McGeer A, Simon AE, Low DE. Epidemiology of needlestick injuries in house officers. *Journal of Infectious diseases* 1990; 162: 961-964.
11. Chia HP, Koh D, Chong R, Jeyaratnam J. A study of needle-stick injuries among house officers in a major hospital. *Singapore Medical Journal* 1994; 35: 41-43.
12. Dalton M, Blondeau J, Dockerty E, Fanning C, Johnston L, LeFort-Jost S, MacDonald S. Compliance with a nonrecapping needle policy. *American Journal of Infection Control* 1992 Summer; 7(2):41-4.
13. English JF. Reported hospital needlestick injuries in relation to knowledge/skill, design, and management problems. *Infection Control and Hospital Epidemiology* 1992 May; 13(5): 259-64.
14. Varma M, Mehta G. Needlestick injuries among medical students. *Journal of Indian Medical Association* 2000 Aug; 98(8): 436-8.
15. Haiduven DJ, De Maio TM, Stevens DA. A five-year study of needlestick injuries: significant reduction associated with communication, education, and convenient placement of sharps containers. *Infection Control and Hospital Epidemiology* 1992; 13: 265-271.
16. Zafar AB, Butler RC, Podgomy JM, Mennonnes PA, Gaydos LA, Sandiford JA. Effect of a comprehensive program to reduce needlestick injuries. *Infection Control and Hospital Epidemiology* 1997; 18(10): 712-715.
17. Gershon RR, Pearse L, Grimes M, Flanagan PA, Vlahov D. The impact of multifocused interventions on sharps injury rates at an acute-care hospital. *Infection Control and Hospital Epidemiology* 1999; 20(12): 806-811.
18. Edmond M, Khakoo R, Mc Taggart B, Solomon R. Effect of bedside needle disposal units on needle recapping frequency and needle-stick injury. *Infection Control and Hospital Epidemiology* 1988 Mar; 9(3):114-6.

Stability of the recombinant hepatitis B surface antigen expression vector in genomic DNA of *Hansenula polymorpha* yeast cells (Master Cell Bank)

*Win Aung, **Suk Hoon Ha, **Jae Seung Kim,
**Yeon Hee Kim & *Khin Pyone Kyi

*Hepatitis B Vaccine Plant

Department of Medical Research (Lower Myanmar)

**Research and Development Centre, Institute of Sciences and Technology
CJ Corporation, Republic of Korea

For production of recombinant hepatitis B (HB) vaccine, fermentation of the transformed *Hansenula polymorpha* yeast cells containing hepatitis B surface antigen (HBs Ag) expression vector stored as lyophilized form (Master Cell Bank) at 4°C followed by several purification steps has to be performed. In our study, the existence of the structural HBsAg gene and URA 3 selective marker gene in the HBsAg expression vector, integrated in chromosomal DNA of recombinant *Hansenula polymorpha* yeast cell was determined. First, genomic DNA of *Hansenula polymorpha* yeast cell was extracted from MCB and purified by using QIAGEN genomic DNA extracting kit, followed by restriction enzyme digestion, PCR amplification, agarose gel electrophoresis and Southern blot hybridization. The nucleotide sequence of HBsAg DNA segment was also confirmed by DNA Genetic Analyser. In addition, the presence of the URA3 marker gene in the HBsAg expression vector was also identified by culturing of transformed *Hansenula polymorpha* colonies on the FOA containing culture plate for their survival. The results indicated that both structural HBsAg expressed gene and URA 3 marker gene were found to be stable in chromosomal DNA of *Hansenula polymorpha* yeast cells at 4°C on long-term storage.

INTRODUCTION

After transfer of technology from the Institute of Science and Technology, CJ Corporation, Republic of Korea, recombinant hepatitis B vaccine was successfully developed by the Hepatitis B Vaccines Plant, DMR (LM) in 2004 by using *Hansenula polymorpha* yeast cells as an effective host system. In this recombinant DNA technology, antigen expression vector (pH-HBs) was prepared by step-wise construction consisting of hepatitis B surface antigen (HBsAg) structural gene (678 base pairs), methanol oxidase (MOX) promoter gene (961 bp), URA 3 marker gene (451 bp), *Hansenula* Autonomously Replicating Sequence (HARS) functional gene (440 bp) and Transcription terminator gene (320 bp). This vector was then transformed and

incorporated into the chromosomal DNA of *Hansenula polymorpha* yeast cells. After cultivation of these cells in selective media, they were freeze-dried followed by aseptic sealing in glass vials which were kept at 4°C for long-term storage as Master Cell Bank (MCB) before used for the production process [1].

In our study, the existence the HBsAg expression vector, integrated in the genomic DNA of *Hansenula polymorpha* yeast cells (MCB) stored at 4°C was determined with an aim to confirm its stability for using as a starting material in process of recombinant HB vaccine production.

MATERIALS AND METHODS

The lyophilized form of MCB containing the recombinant HBsAg expressed *Hanse-*

nula polymorpha yeast cells, stored at 4°C for about 4 years, a product of CJ Corporation, Republic of Korea was first reconstituted with 1 ml of autoclaved distilled water. The suspension was smeared on 0.7 % Yeast Nitrogen Base (YNB) culture plate which was kept at 30°C incubator for 48 hours. On day 3, a single colony from the above culture plate was inoculated into 5 ml of 0.7 % YNB broth, with 2 % glucose and it was then incubated at 30°C with 250 rpm for 24 hours.

1. Identification of HBsAg expressed gene

From the above cultured broth containing transformed *Hansenula polymorpha* yeast cells, a genomic DNA was extracted and purified by using the QIAGEN Genomic DNA Extraction kit [2].

1.1. Southern blot identification

The extracted whole chromosomal DNA segment was digested by Restriction Enzyme Dra I. Then, the cut DNA fragments were identified by using agarose gel electrophoresis. Finally, this HBsAg expressed gene was confirmed by Southern Hybridization by using a specific probe prepared with specific primers; AY 21 F (5'- ATTGAGAACATC ACATCAGGA-3') and AY 21 R (5'-TTGG TAACAGCGG TAAAAAGGGAC.3') in PCR, binding with sequence of recombinant HBsAg gene labeled with digoxigenin-dUTP.

1.2. DNA sequencing analysis

The extracted genomic DNA containing the gene of interest was also amplified by thermocycler using specific primers: AY 21 F (5'- ATGGAGAACATCACATCAGGA-3') and CTR (5'-CTCTTTGTTTTGTTAGGGT-3'). Then, DNA segments were purified by using PCR purification QIAGEN test kit [3]. The purified HBsAg gene was confirmed by agarose gel electrophoresis, and nucleotide sequencing analysis was performed by using the ABI Prism 3100 Genetic Analyzer [4].

2. Determination of URA 3 marker gene

Serial dilutions (i.e. 10^{-2} , 10^{-4} , 10^{-6} & 10^{-8}) of the above culture in 0.7% YNB broth

were done with distilled water and smeared on Yeast Peptone Dextrose (YPD) culture plates. After 48 hours incubation at 30°C, 100 colonies were picked up from these plates and transferred on to the YPD plates containing 5-Fluoroorotic acid (5-FOA) and also on to the YPD plate without 5-FOA. After cultivation of these plate at 30°C for 48 hours, number of viable colonies in each culture plate media was counted.

RESULTS

Figure 1 shows the agarose gel electrophoretic pattern of chromosomal DNA segments extracted from MCB, cut by Restriction Enzyme Dra I. Many partially cut DNA segments were observed indistinctly along the lane of digested test sample whereas uncut (i.e. undigested control) whole DNA segment was detected at the level of 12 Kb pairs. Southern hybridization of these DNA segments by using specific probe revealed that distinct DNA band of HBsAg expression vector containing the HBsAg structural gene (~700 bp) and part of MOX promoter gene (~400 bp) were observed at the level of 1.1 Kbp(cut) and 12 Kbp (uncut) indicating the existence of the HBsAg expression vector in genomic DNA of *Hansenula polymorpha* yeast cell (Fig. 2).

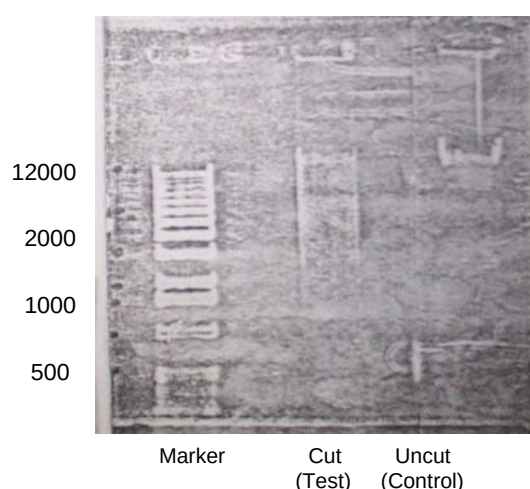


Fig.1. Agarose gel electrophoretic pattern of chromosomal DNA segment of *Hansenula polymorpha* yeast cell, digested by Restriction Enzyme Dra I

Agarose gel electrophoretic identification of the HBsAg gene extracted from the chromosomal DNA of *Hansenula polymorpha* yeast cells amplified by specific primers followed by PCR purification is illustrated in Fig 3. The distinct DNA band was observed at about 700 base pair level totally identical to that of the theoretical HBsAg gene, confirming the identity between number of base pair of HBsAg gene in expression vector and that of the gene of interest.

Figure 4 represents the nucleotide sequence of the HBsAg DNA segment, extracted from the chromosomal DNA of *Hansenula polymorpha* yeast cells analysed by using the genetic analyzer. The nucleotide sequences obtained were found to be totally identical to that of the HBsAg gene.

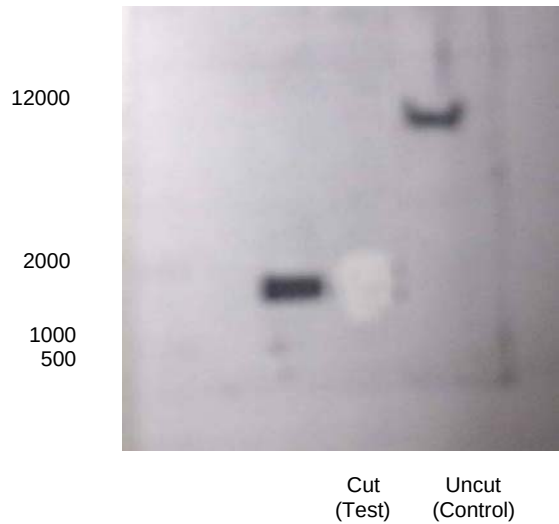


Fig .2. Southern hybridization of chromosomal DNA segments after digestion with RE Dra I by using a specific probe

Figure 5 depicts the growth of transformed *Hansenula polymorpha* colonies cultured on YPD plate with 5-FOA and plate without 5-FOA. All colonies cultured on YPD without FOA plate showed 100 % survival where as those cultured on YPD plate containing 5-FOA showed no survival. It indicated the coexistence of URA 3 functional gene with the HBsAg structural gene in the plasmid DNA segment integrated in genomic DNA of *Hansenula polymorpha* yeast cell.

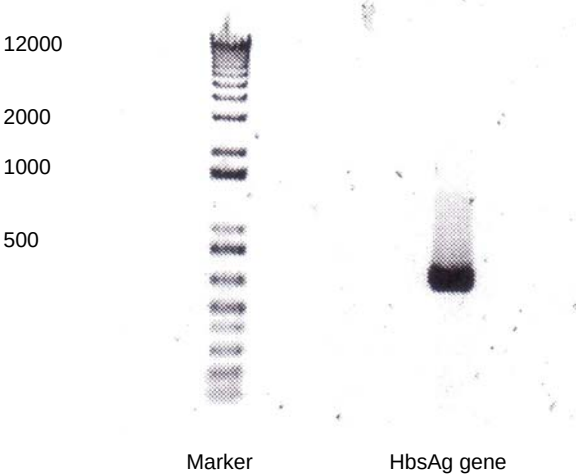


Fig. 3. Agarose gel electrophoretic identification of the HBsAg gene extracted from the chromosomal DNA of *Hansenula polymorpha* yeast cells

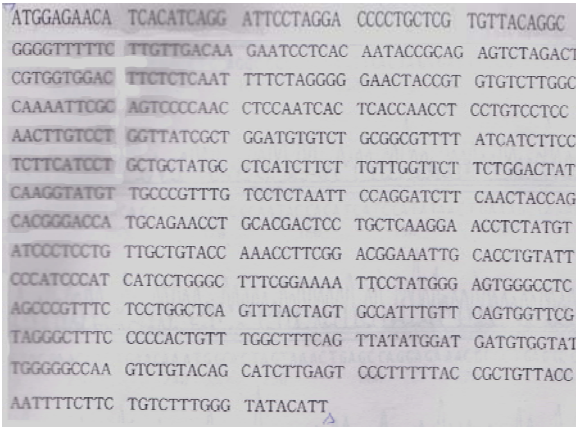


Fig. 4. Nucleotide sequence of the HBsAg DNA segment, extracted from the chromosomal DNA of *H. polymorpha* yeast cells

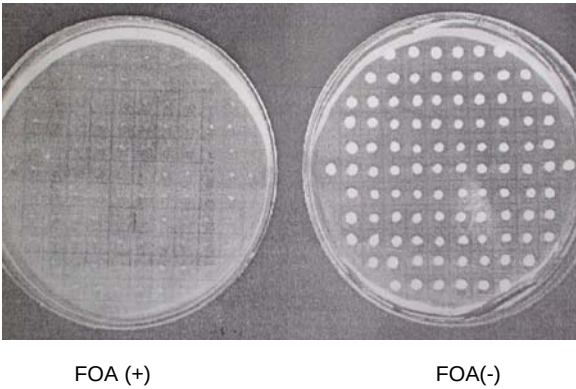


Fig. 5. Growths of transformed *Hansenula polymorpha* colonies in FOA containing media and FOA free media

DISCUSSION

It is well documented that *Hansenula polymorpha* yeast cell is the latest and efficient expression system for manufacturing recombinant HB vaccine. It is highly acceptable for mass production because of its preference to massive cultivation and control of protein expression from the recombinant *Hansenula polymorpha* yeast cell under control of MOX promoter gene. In addition, since the expression vector is incorporated in the chromosomal DNA, it is more stable than that of other vectors which are extra-chromosomal after transformation [5].

Because of the lack of orotidine 5-phosphate decarboxylase (OPD) which controls the uracil synthesis in *Hansenula polymorpha* yeasts, wild type (i.e untransformed) cells can not survive in uracil free- media. The plasmid of the transformed these yeast cell contains URA 3 gene which controls uracil synthesis in cells by expression of OPD. It transforms 5-fluro orotic acid (5-FOA) into cytotoxic 5 fluro uracil (5 FU). Therefore, transformed *Hansenula polymorpha* yeast cell containing HBsAg expression vector can not survive on media containing 5-FOA [1]. Based on these characteristics, stability of URA 3 gene was evaluated in our study by culturing of transformed colonies on FOA containing media and FOA free media. Our finding showed that all transformed cells could not survive on media containing FOA, thus indicating the existence of URA 3 maker gene in the HBs Ag expression vector of *Hansenula polymorpha* yeast cell.

This study also showed that, both HBsAg structural gene and MOX maker gene were identified in the expression vector integrated in genomic DNA of *Hansenula polymorpha* yeast cell. In addition, studying the stability of HBs Ag gene in *Hansenula polymorpha* yeast cells, the existence of the gene of interest was indicated by agarose gel electrophoresis followed by Southern hybridization as well as by nucleotide sequencing analysis.

In Myanmar, the HB Vaccine Plant, DMR (LM), Ministry of Health has been producing recombinant HB vaccine after transfer of technology from CJ Corporation, Republic of Korea since 2004. The lyophilized form of MCBs (i.e. transformed *Hansenula polymorpha* yeast cell) containing HBsAg expression vector provided by CJ corporation was used as a starting material for the fermentation and purification processes of HB vaccine production. It is necessary to determine the existence and stability of HBsAg expression vector in MCB periodically (i.e. once a year or prior to each and every batch of production process) [6].

The results of our study showed that the structural HBs Ag gene, MOX promoter gene and URA3 marker gene were identified in MCB containing transformed *Hansenula polymorpha* yeast cells. Therefore it could be concluded that the recombinant HBsAg expression vector was totally integrated and stable in genomic DNA of *Hansenula polymorpha* yeast cell (lyophilized form) kept at 4°C for long-term storage, indicating that MCBs could be used effectively and safely as a starting raw material for production of recombinant HB vaccine in the HB Vaccine Plant, Myanmar.

REFERENCES

1. CJ Pharmaceutical Company (2000). Safety and Effectiveness of CJC 50100, hepatitis B vaccine (Recombinant), *Product Manual*, Republic of Korea. 2000; 1- 55.
2. *Handbook of the QIAGEN Genomic DNA* 1995; 1-50.
3. *User's guide of the Bioneer AccuPrep Plasmid Extraction Kit* 1999: 1-13.
4. *Sequencing Chemistry Guide for ABI Prism 3100 Genetic Analyzer*. HITACHI. 1-21.
5. Barnett JA, Payne RW & Yarrow D. In: *Yeasts: characteristics and identification*, published by the Press Syndicate of the University of Cambridge USA 1983; 7-17.
6. Requirements for hepatitis B vaccines made by recombinant DNA technology. *WHO Technical Report Series* 1989; no. 786:38-60.

Detection of low level viral genome (HBV DNA) by Polymerase Chain Reaction in nonresponders of plasma-derived hepatitis B vaccine produced at the Department of Medical Research (Lower Myanmar)

Moh Moh Htun, **Khin Pyone Kyi, **Khin May Oo, ***San San Oo, ***Khin Ohmar Lwin & *****Kenji Abe*

**Pathology Research Division*

***Experimental Medicine Research Division*

****Blood programming Division*

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

******National Institute of Infectious Diseases, Japan*

Myanmar is an area of high endemicity for hepatitis B with a 35-60% infection rate and 10-15% HBsAg carrier rate. Several vaccine trials carried out at the Department of Medical Research (Lower Myanmar) using plasma-derived hepatitis B vaccine have shown that there was approximately 10% non-responder rate. Out of 337 apparently healthy persons who were initially screened for HBsAg, antiHBs and antiHBc by using the Abbott ELISA test kits (USA), 137 persons were negative for all markers (HBsAg, AntiHBs and AntiHBc). They were immunized with three doses of locally produced plasma-derived hepatitis B vaccine (10µg/ml) by intramuscular route in the deltoid region. One month after the completion of three doses of hepatitis B vaccine, 38 subjects who did not respond were vaccinated again using the same dose, site and route. Out of 38 vaccinees, 23 subjects did not have detectable levels (10 m IU/L) at two months after the booster vaccination. HBV DNA was extracted from all serum samples of nonresponders by using the phenol, chloroform and ethanol method. These extracted samples were tested for HBV DNA by using the Polymerase Chain Reaction (PCR). After the amplification of 40 cycles, HBV DNA was detected by 2% agarose gel electrophoresis. The short nucleotide of the HBV DNA (< 200bp) of the positive two samples were amplified successfully by nested PCR and sequenced by Taq Dye Deoxy TM Terminator Cycle Sequencing Kit. These two nonresponders (8.69%) may be due to silent HBV infection.

INTRODUCTION

Myanmar is an area of high endemicity for hepatitis B with 35-60% infection rate [1] and 10-15% HBsAg carrier rate [2]. In Myanmar, the control of viral hepatitis is one of the top priority programmes in the National Health Plan (NHP). Hundreds of millions of plasma-derived hepatitis B vaccines have been used worldwide since 1981 with an outstanding record of safety and efficacy. Several vaccine trials carried out at the Department of Medical Research (Lower Myanmar) using plasma-derived

hepatitis B vaccine have shown that there were approximately 10% non-responder rates after three doses of vaccine. Genetic factors may play a role in non-responsiveness to HB vaccine in some instances and low level HBV related viral infection may be another possible mechanism to explain this phenomenon [3]. Hepatitis B virus is a member of a family of DNA virus called Hepadnaviridae [4]. Its genome consists of approximately 3200 base pairs and contains various genes: PreC/Cgene codes for two viral components, polypeptides making up the nucleocapsid

and containing the HB core antigen (HBcAg) and a protein which becomes measurable in the blood after post translation process as HBeAg. PreS1/PreS2/S gene make up the viral envelope which contains HBsAg. The P gene codes for the viral DNA polymerase which is central for viral reproduction. X gene is thought to exert various regulatory functions for the virus [5].

Although the serological markers have been shown to be sensitive and convenient for detecting the HBV infection, they are not always good indicators of viral activity. Viral DNA is a more reliable and direct evidence of the presence of the virus [6, 7, 8, 9]. Polymerase Chain Reaction is a highly sensitive and specific method, which can detect a single copy of a gene (HBV DNA) [10]. It is at least 10^4 times more sensitive than dot blot hybridization assays for HBV DNA [11].

Objective

- To detect low levels of (HBV DNA) in non-responders of plasma-derived hepatitis B vaccine produced at Department of Medical Research (Lower Myanmar) using the Polymerase Chain Reaction technique.

MATERIALS AND METHODS

A total of 668 apparently healthy persons comprising of 428 males and 240 females were initially screened for liver function test and HBsAg, antiHBs and antiHBc. Among them, 289 subjects were negative for HBsAg, antiHBs and antiHBc. They were immunized with three doses of plasma-derived hepatitis B vaccine (10 µg/ml) produced at the Department of Medical Research (LM) by intramuscular route in the deltoid region. One month after the completion of three doses, 251 persons developed antibody to hepatitis B (>10 IU/L of antiHBs) as tested by Abbott ELISA kit and the remaining 38 vaccinees who did not respond to vaccination were immunized with 10µg/ml of the same vaccine as booster

dose intramuscularly at the same site. Twenty-three vaccinees out of 38 did not have detectable level (10 IU/L) at two months after the booster vaccination. HBV DNA was extracted from these 23 serum samples by the phenol, chloroform and ethanol method. Briefly, 100 µl of serum was incubated at 70°C for 3 hours in 300 µl of 1% SDS containing 15mM Tris HCl (pH = 8), 10mM EDTA (pH = 8) and 500 µg/ml of proteinase K (Merk, Darmstadt, Germany).

The nucleic acids were purified by phenol, chloroform extractions followed by precipitation with isopropanol. The resulting pellet was resuspended in 50 µl of RNase-free water, then 4 µl of this solution was used as a template for HBV DNA amplification. The 40 µl of reaction buffer was made up for PCR1 which contained 10 x Gold buffer (1 x buffer containing 1.5mM MgCl₂), 5 U Ampli Taq Gold DNA polymerase (Perkin-Elmer, Norwalk, CT, USA), dNTP mix (10mM) and two pairs of PCR primers (MD24 and MD26 / HBX1 and HBX2). Thermocycler was programmed first to incubate the samples for 50 min at 37°C, then preheat at 95°C for 10 min to activate Ampli Taq Gold followed by 40 cycles consisting of 94°C for 20 seconds, 55°C for 20 seconds and 72°C for 7 minutes using a Perkin-Elmer 9700 Thermal Cycler (Perkin-Elmer). For the PCR 2 reaction, 2 µl of the first PCR product was mixed with same PCR buffer and second set of each inner primer. Thermocycling for 40 cycles was done as above but the annealing temperature was set at 60°C instead of 55°C for the second round of PCR [12].

The PCR products were electrophoresed on 2% agarose gels staining with ethidium bromide and evaluated under UV light. The sizes of PCR products were estimated according to the migration pattern of 50bp DNA ladder (Pharmacia Biotech, Piscataway, NJ, USA). The two serum samples (NR 16 & 18) which were positive for HBV DNA were purified by using the QIA quick PCR purification kit

(Germany). The recovered PCR products were subjected to direct sequencing from both directions using an ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer). Sequences of amplified cDNA were determined using a sequencer (ABI model 310 Genetic Analyzer) according to computer programme.

Primer sequences used for nested PCR

Region of gene for HBV DNA	Level of PCR	Name of primer	Nucleotide sequences	Product size
X region	PCR 1	MD 24	5'TGCCAACTGGATCCTTC GCGGGACGTCCTT-3' (nt 1392-1421)	233bp
		MD 26	5'GTTACAGGTGGTCTCC ATG-3' (nt 1625-1607)	233bp
X region	PCR 2	HBX1	5'GTCCCCTTCTCATCTG CCGT-3' (nt 1487-1507)	117bp
X region	PCR 2	HBX2	5'ACGTGCAGAGGTGAAG CGAAG-3' (nt 1604-1584)	117bp

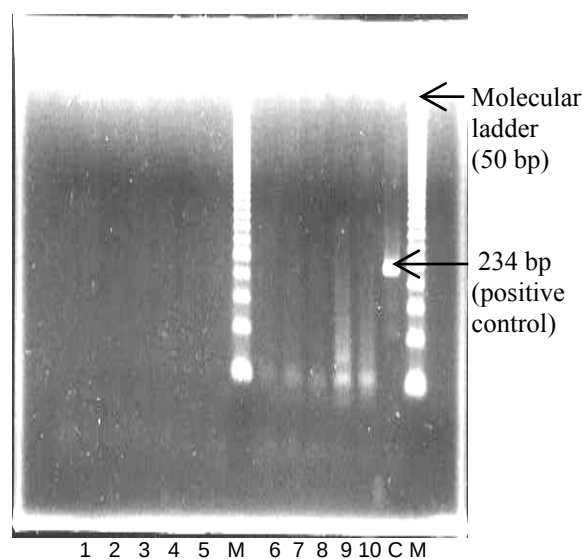


Fig. 1. Detection of HBV DNA in 10 samples from nonresponders amplified by PCR1 reaction, analyzed by 2% gel electrophoresis with ethidium bromide staining

In all samples, the multiple non-specific bands were detected according to 50 bp marker ladder. HBV DNA in positive control band was specifically detected at 234bp (arrow) (Fig.1). At 7 and 8 samples, a distinct band at the same level as positive control (118bp) was seen. The other samples

of nested PCR products did not show the specific band (Fig.2).

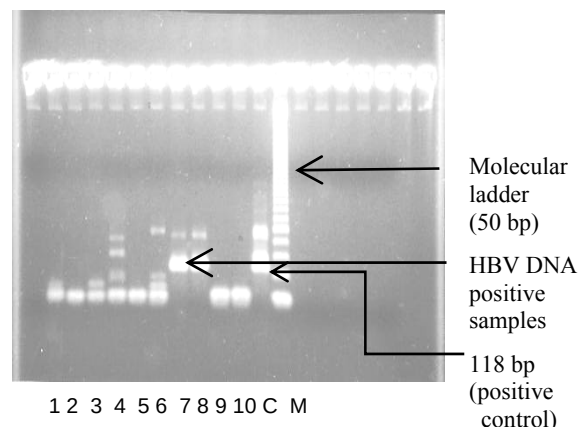


Fig. 2. Detection of HBV DNA in 10 samples of amplified nested PCR products analyzed by 2% agarose gel electrophoresis with ethidium bromide staining

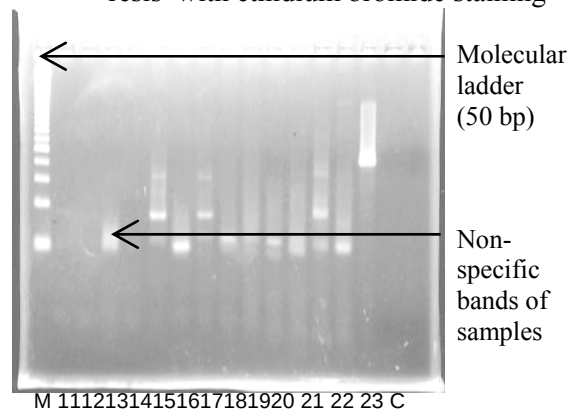


Fig. 3. Detection of HBV DNA in the last 13 samples of amplified PCR 1 products analysed by 2% gel electrophoresis with ethidium bromide staining

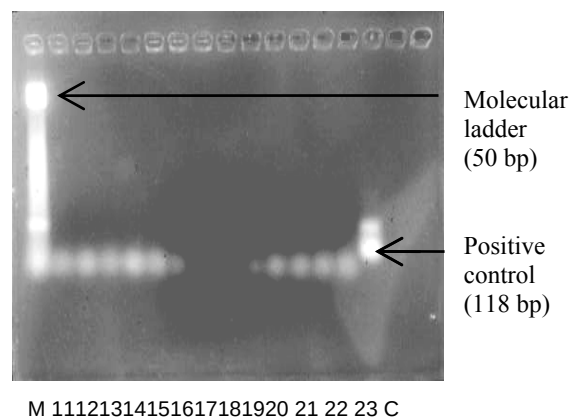


Fig. 4. Analyzing of last 13 samples of nested PCR 2 products by 2% agarose gel

In all serum samples were detected multiple nonspecific band at different levels according to the 50 bp marker ladder. The typical band of 234 bp was only detected in the positive control in this study (arrow) (Fig.3) The specific band was not detected in all 13 samples of non-responders at the same level of positive control (118bp) according to the marker ladder (50bp) (arrow) (Fig.4).

RESULTS

Two hundred and eighty nine apparently healthy persons received three doses of DMR plasma-derived hepatitis B vaccine, out of which 38 persons did not respond to this vaccine at one month after the completion of three doses and 23 non-responders did not have detectable level (10mIU) at two months after the booster vaccination. Persistence of non-responders rate (%) was highest in > 61 years age group (100%) and 51-60 years age group (85.71%). Lowest nonresponders rate was found in 11-20 year age group (0%) and 21-30 years age group (40%). The non-responders rate was 57.41% in 31-40 years age group and 60% in 41-50 years age group (Table 1). The non-responder persistence rate was 64.51% in male and 42.85% in female after booster vaccination (Table 2). After extraction of DNA from each serum sample of nonresponders by using the phenol/ chloroform method, non specific bands of first 10 samples were found in PCR 1 which was analyzed by 2% agarose gel electrophoresis stained with ethidium bromide. HBV DNA positive control band (234bp) was detected according to 50bp marker ladder (Fig.1). The distinct bands of two serum samples (NR 16 and NR 18) were found at the same level of positive control band (118bp) after amplification of nested PCR 2 using 2% agarose gel electrophoresis stained with ethidium bromide. The other samples did not show specific band on gel (Fig. 2). The remaining DNA extraction of 13 serum samples showed nonresponders bands according to the marker ladder (50bp)

but positive control band (234bp) was found in 2% agarose gel electrophoresis stained with ethidium bromide after amplification of PCR1 (Fig. 3). The product of second nested PCR2 did not show the specific bands in 2% agarose gel, although 118bp positive control was detected according to 50bp marker ladder (Fig. 4).

The short nucleotide sequence of the HBV DNA (>200bp) of the positive two samples (NR 16 and NR 18) were amplified successfully by nested PCR and directly sequenced by using the Taq Dye DexoyTM Terminator cycle kit. This different four colors pattern of nucleotide sequences (adenine, guanine, cytosine and thiamine) of two amplified nested PCR 2 products came out of from sequencer (ABI model 310 Genetic Analyzer) according to the primer pairs used at PCR 2 (HBX 1 and HBX 2).

Table 1. Non-responders of DMR plasma-derived hepatitis B vaccine in relation to age

Age (years)	Non-responders before booster vaccination			Non-responders after booster vaccination			% of non-responder
	M	F	Total	M	F	Total	
10-20	1	-	1	-	-	-	0
21-30	3	2	5	2	-	2	40.00
31-40	12	2	14	7	1	8	57.14
41-50	9	1	10	6	-	6	60.00
51-60	6	1	7	5	1	6	85.70
>61	-	1	1	-	1	1	100.00
Total	31	7	38	20	3	23	

M=Male F=Female

Table 2. Non-responders of DMR plasma-derived hepatitis B vaccine in relation to sex

Sex	Total number of non-responders	Non-responders after booster vaccination	% of non-response
Male	31	20	64.51
Female	7	3	42.85

DISCUSSION

In this study, out of 38 nonresponders, twenty-three subjects did not respond to booster vaccination of plasma-derived

hepatitis B vaccine after interval of six months from first vaccination. The results showed that the lowest antibody response was found in > 50 years age group and highest response was seen in < 30 years age group. Neonates and young infants generally respond very well to the vaccine, in contrast to older patients who tend to have a diminished immune response [13].

There was a significant correlation between the antibody response and age of the vaccinees in this study. In a previous human immunogenicity trial, using plasma-derived hepatitis B vaccine, the immune response as evidenced by presence of anti HBs, was seen in 90.16% in males and 89.74% in females vaccinees [14]. There was no significant correlation between antibody response and sex in this study (64.51% in males and 42.85% in females).

Certain risk factors such as increasing age, male gender, and obesity, history of smoking and administration of vaccine in the buttock rather than deltoid muscle were some of the risk factors associated with decreased rates of sero-conversion [15]. Low level HBV DNA was detected in the sera of two out of 23 nonresponders, by the Nested Polymerase Chain Reaction (Nested PCR) although hepatitis B markers (HBsAg, antiHBs and antiHBc) were not detected in all the sera samples of the nonresponder when tested by the ELISA method (Abbott EIA). ELISA assay could detect the HBsAg 100-200 pg/ml of serum, corresponding to roughly 3×10^7 particles /ml. This method is not a perfect indicator for hepatitis B infection especially in endemic region. The calculated weight of HBV DNA equivalent to one virion is approximately 3×10^6 pg. Dot blot assay may detect at least 10^{3-5} virions of one genome equivalent per sample. (3×10^6 pg) [16]. Serum hepatitis B virus (HBV DNA) was detected in eight subjects (7.3%) out of 107 HBsAg negative normal Chinese subjects by using polymerase chain reaction which showed that serological markers were not adequate to rule out HBV infection [17].

In this study, the short nucleotide sequence (117bp) of X gene was detected in two subjects (NR 16 and NR 18) by nested PCR method and sequenced by Taq Dye DeoxyTM Terminator cycle sequencing kit and ABITM gene analyser model 310. HBV mutant was responsible for the pathogenesis of "silent" hepatitis B in patients who were negative for both hepatitis B surface antigen (HBsAg) and anti-hepatitis B core antibody (antiHBc) [18]. HBV DNA alone was detected in silent hepatitis B infection in the absence of hepatitis B markers. These two nonresponders (NR16 and NR18) may be due to silent hepatitis B virus infection in this study. Silent HBV infection could be explained by the 8 nucleotide deletion and DR 2 mutation in the X gene coding region. These mutations suppressed the replication and expression of HBV DNA, leading to low levels of HBsAg and AntiHBc which could not be detected by conventional immunological assay techniques [19].

REFERENCES

1. Khin Pyone Kyi, Than Swe, Khin Mya Lwin, Khin Yee Oo & Soe Lwin. Prevalence of hepatitis B in health care personals. 25th Myanmar Medical Conference 1988.
2. Khin Maung Tin, Tin Htut, Hla Myint & Tun Khin. Prevalence of hepatitis A, B in Myanmar. 1st Conference of Medical Speciality 1981; 26-31.
3. Takahashi H, Liang JT, Hubert E, Zeniya M, et al. Identification of low level hepatitis B viral genome in hepatitis B vaccine nonresponders in Japan. In: *Viral hepatitis and liver diseases*. Hollinger FB, Lemon SM & Margolis HS, eds. Baltimore, Maryland, Williams & Wilkins. 779-781.
4. Hollinger FB. In: *Hepatitis B Virus Field Virology*, 3rd edition, edited by Fields BN, Knipe DM, Howley PM et al., Lippincott-Raven Publishers, Philadelphia 1996.
5. Grob PJ. Hepatitis B virus, pathogenesis and treatment. *Vaccine* 1998; 16: S11-16.
6. Banino F, Hoyer B, Nelson J, Engle R, Verme G, & Gerin J. Hepatitis B virus DNA in the sera of HBsAg carriers: a marker of active hepatitis B virus replication in the liver. *Hepatology* 1981; 1: 386-391.
7. Brechot C, Pourcel C, Hadchouel M, Dejean A, Louise A, Scott J & Tiollais P. State of hepatitis

- B virus DNA in liver diseases. *Hepatology* 1982; 2: 27-34.
8. Liberman HM, Labrecque DR, Kew MC, Hadziyannis SJ & Shafritz DA. Detection of hepatitis B virus DNA directly in human serum by a simplified molecular hybridization test: comparison to HBsAg/ antiHBe status in HBsAg carriers. *Hepatology* 1983; 3: 285-291.
 9. Scotto J, Hadchouel M, Hery C, Yvart J, Tiollais P & Brechot C. Detection of hepatitis B virus DNA in serum by a simple spot hybridization technique: comparison with results for other viral markers. *Hepatology* 1983; 3: 279-284.
 10. Theirs V, Nakajima E, Kremsdorf D, Mack D, Schellekens H & Driss F, *et al.* Transmission of hepatitis B from hepatitis B seronegative subjects. *Lancet* 1988; 2: 1273-1276.
 11. Dusheiko G, Xu J, & Zuckermann AJ. Clinical Diagnosis of Hepatitis B infection; Application of the Polymerase Chain Reaction. Y. Becker G. Darai (eds). *Diagnosis of human viruses by PCR technology. Frontiers of Virology* 1. 72.
 12. Abe K, Konomi N & Abdel-Hamid M. Direct PCR for detection of hepatitis B, C and G virus genomes from serum without nucleic acid extraction - simple, rapid and highly sensitive method. *Hepatology Research* 1998; 13: 62-70.
 13. Denis F, Mounier M, Hessel L *et al.* Hepatitis B vaccination in the elderly (letter). *Journal of Infectious Diseases* 1984; 149: 1019.
 14. 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 (LM).
 15. Hollinger FB. Factors influencing the Immune response to hepatitis B vaccine, booster dose guidelines, and vaccine protocol recommendations. *American Journal of Medicine* 1989; 87: 3A 36s-41s.
 16. Yokosuka O, Omata M, Hosada K, Tada M, Ehata T & Ohto M. Detection and direct sequencing of hepatitis B virus genome by DNA amplification method. *Gastroenterology* 1991; 100: 175-181.
 17. Shih LN, Sheu JC, Wang JT, Huang GT, *et al.* Serum hepatitis B virus DNA in healthy HBsAg negative Chinese adults evaluated by Polymerase Chain Reaction. *Journal of Medical Virology* 1990; 32: 257-260.
 18. Saito T, Shinzawa H, Uchida T, Kawamata O, Honma S, *et al.* Quantitative DNA analysis of low level hepatitis B viremia in two patients with serologically negative chronic hepatitis B. *Journal of Medical Virology* 1999; 58: 325-331.
 19. Uchida T, Gotoh K & Shikata T. Complete nucleotide sequences and characteristics of two hepatitis B virus mutants causing serologically negative acute or chronic hepatitis B. *Journal of Medical Virology* 1995; 45: 247- 252.

Antimalarial activity and related chemical constituents of *Swertia* species from Kayah State

*Khin Phyu Phyu, **Win Myint, ***Ye Htut, ****Mya Aye,
***Kyin Hla Aye & ***Ni Ni

*Biochemistry Research Division

Department of Medical Research (Upper Myanmar)

**Department of Traditional Medicine (Nay Pyi Taw)

***Department of Medical Research (Lower Myanmar)

****University of Mandalay

The most promising activity was found in ethanol extracts of *Swertia purpurescens* (whole plant) at the dosage of 2.8 g/kg/day both in suppressive and therapeutic tests when various extracts of these three plant specimens were preliminary screened for antimalarial activity, using in *in vivo* model. The active ethanol extract observed to have significant antimalarial activity in *in vivo* model was subjected to *in vitro* *p. falciparum* system. It's antimalarial activity was found starting from the dosage of 1000 µg/ml. Chemical separation of the active extracts was serially carried out by means of solvent-solvent partition, column chromatographic and thin layer chromatographic techniques. Out of seven components yielded, only one showed to have suppressive activity against malaria parasite in both experimental models. The active compound was identified as a xanthone molecule by means of UV, FTIR, ¹HNMR (600MHz), DQF-COSY, HSQC, HMBC and NOE spectroscopic techniques. *Swertia chirata* and xanthone molecules have known to be antimalarial agents in recent years. Therefore, this study could structurally elucidate an active compound Bellidifolin from *S. purpurescens* and for the first time, the possessing of antimalarial activity in *S. purpurescens* could be reported.

INTRODUCTION

Plant-based Antimalarial drugs such as quinine and artemisinin were discovered in the natural product recourse area. Medicinal plants and the knowledge of traditional medicine are the prime recourses for the development of anti-malarial agents. In Myanmar, it is observed that traditional and herbal medicine are used and heavily relied upon by most people for health purposes. Therefore, a study was conducted on that plant to investigate the chemical constituent and to evaluate its activity for further development if a useful anti-malarial agent derived from Myanmar plant sources.

This paper represents a critical account of crude extracts and the active constituents

with the chemical structure possessing antimalarial activity against *P. falciparum* and *P. berghei* experimental models.

MATERIALS AND METHODS

Preparation of plant extracts and phyto-chemical tests

Three species of the same genus commonly known as Pan Kha were collected from Loikaw in November because it was reported by the local people that the plant was most active in the specified season. These species were identified and confirmed by their specific botanical names by a competent taxonomist from the Department of Botany, Mandalay University. The whole plant was used for the chemical extraction.

Each plant species was extracted successively with the solvents such as chloroform, ethanol and water to yield nine extracts. Preliminary phytochemical investigations were carried out for three plants to determine the presence of the organic constituents (Physico-chemical standard of Unani Medicine) [1].

Acute toxicity test

Ten albino mice in each group were fasted overnight before administration of each plant extract. Increasing doses of three species of chloroform extracts, 0.6, 1.2 and 2.4g/kg and alcohol and watery extracts, 0.7, 1.4 and 2.8 g/kg were orally administered. The mice were housed separately in individual cage with free access to food and water and observed clinically for 1 week. LD₅₀ of three plants extracts were determined after performing the acute toxicity test [2].

Screening of plant extracts for antimalarial activity in in vivo and in vitro model

Plasmodium berghei and Dutch Denken Yoken (DDY) mice for *in vivo* testing and *Plasmodium falciparum* parasites for *in vitro* testing were used. Nine extracts were initially screened for antimalarial activity by applying *in vivo* model using *P. berghei*. Both suppressive and therapeutic tests were performed [3, 4]. One of the ethanol extract which showed outstanding antimalarial activity in both *in vivo* tests, was subjected to *in vitro* during testing system [5]. To be able to attain homogeneous solution, chloroform extracts of all three plants were dissolved in olive oil. Forty percent alcohol was used to dissolve alcohol extracts in *in vivo* models. Aqueous extracts were easily dissolved in water. For *in vitro* experiments, the ethanol extract which was found to be the most active ingredient was dissolved in RPMI medium. Twenty percent alcohol was used to produce homogeneous solution of ethanol extract in *in vitro* models.

Chemical separation of active extract and confirmatory test for antimalarial activity

The active extract which appeared in both *in vivo* and *in vitro* experiments was further

analysed to isolate the individual chemical constituents. Preliminary investigation was carried out by thin layer chromatography (TLC) [6] with the solvent system of hexane: ethyl acetate (7:3). On the basis of TLC findings, column chromatographic separation was further carried out by using hexane and ethyl acetate (9:1, 7:1, 6:1, 4:1, 7:3, 3:2, 1:1) as eluent. Thus a sufficient amount of the major constituent form, this active extract was obtained for further chemical analysis.

Chemical structure elucidation

Literature background [7, 8] was initially reviewed to find out the main class of chemical compounds present in the plant of the genus *Swertia*. It is found to be xanthone as the main compound by means of FTIR, ¹HNMR (600 MHz) and NOE spectronic techniques.

RESULTS

Botanical investigation revealed, three species namely – *Swertia affinis*, *Swertia angustifolia* and *Swertia purpurescens*. The yield percentage of chloroform were 3.85 %, 6.42 %, 5.14%, 95% ethanol extracts were 15.25 %, 18.98 %, 16.67 % and water extracts were 5.49 %, 6.45 %, 11.28 % from *Swertia affinis*, *Swertia angustifolia* and *Swertia purpurescens*, respectively.

According to acute toxicity test conducted on experimental mice, none of the nine extracts produced toxicity if given in suppressive as well as therapeutic dose. LD₅₀ values of chloroform extracts of *Swertia purpurescens*, *Swertia angustifolia* and *Swertia affinis* were the same ie, 2.4g/ kg/ day. The other two, ethyl alcohol and aqueous extracts of these three species were the same ie. 2.8g/kg/day.

Phytochemical test showed glycoside, carbohydrate, flavonoid, polyphenol, steroid, terpene, saponin, lipophilic group and volatile oil were highly positive but alkaloid, protein and amino acid were not present in three species.

In vivo suppressive test, therapeutic test and *in vitro* test

Preliminary screening of different extracts of all medicinal plants were done by performing *in vivo* suppressive test (Table1).

Table 1. *In vivo* parasite suppression of different extracts of three *Swertia* species on day 4 observed in suppressive test

(gm/ kg/ day)	Percent parasite suppression on day 4		
	<i>S.purpurescens</i>	<i>S.angustifolia</i>	<i>S.affinis</i>
Chloroform extract			
2.4	47.19	33.69	36.05
1.2	38.30	15.74	34.19
0.6	25.16	5.96	32.61
Ethanol extract			
2.8	65.2	55.3	34.7
1.4	51.48	41.65	33.62
0.7	44.74	41.65	Nil
Aqueous extract			
2.8	15.28	5.5	13.99
1.4	14.52	Nil	Nil
0.7	8.56	Nil	Nil

The experimental results were expressed in terms of percent parasite suppression. Percent parasite suppression means percentage of parasitaemia reduced in treated group of mice due to tested drug compared to that of untreated control of mice. Percent parasite suppression was determined on Day 4. Ethanol extract in varying doses namely, 0.7g/ kg/day, 1.4g/ kg/ day and 2.8g/ kg/ day showed suppressive effect in all three *Swertia* species, being highest suppression (44.74% to 65.20%) in *Swertia purpurescens*. Therapeutic effect of ethanol extract of three *swertia* species on *Plasmodium berghei* were investigated (Fig.1) and *Swertia purpurescens* extracts gave 44% suppression and *Swertia affinis* gave 31.38% suppression. Therapeutic activities of 95% ethanol extract of *Swertia purpurescens* was significantly more than those of two *Swertia* species ($p<0.5$).

Therefore, the authors chose 95% ethanol extracts of *Swertia purpurescens* for structure elucidation whereas watery and

chloroform extract showed no suppressive activity in *in vivo* models.

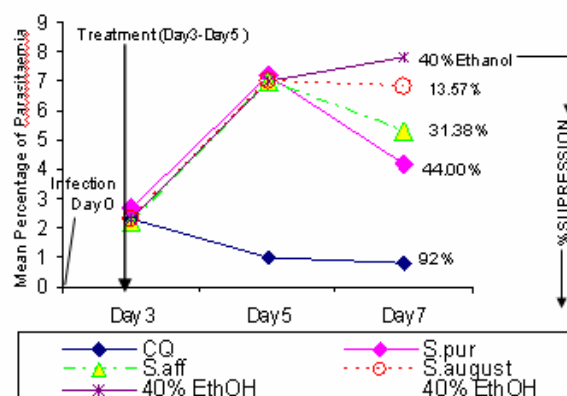


Fig. 1. Therapeutic effect of ethanol extracts of 3 species of *Swertia* plant on *P. berghei* infection in mice

For the chemical separation, the active ethanol extract of *Swertia purpurescens* was dissolved in ethyl acetate solution to provide non polar and polar fractions. These two fractions were again tested in *in vivo* model and it was found that only ethyl acetate soluble fraction was active as shown in Fig. 2.

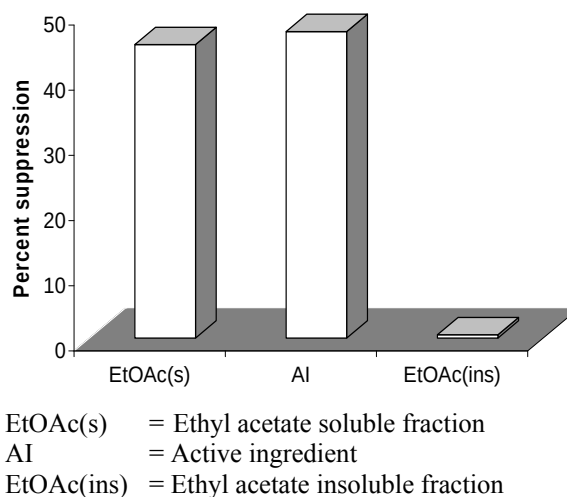


Fig. 2. Parasite Suppression (%) of different fractions of *S. purpurescens* tested in mice

In the *in vivo* experiment, the activities of both ethanol extract and ethyl acetate fraction were found to be active with the dosage of 2.8 g/kg/day and 0.7784 g/kg/day

respectively. Out of seven fractions tested, the *in vitro* results showed that fraction 3 possessed highest activity with ED₅₀ of 175 µg/ml. The TLC study of the most active ethyl acetate soluble fraction of ethanol extract indicated two major spots together with several minor spots under UV light.

Structure elucidation of compound

Among the six possible xanthone structures (Fig. 3) which had been already reported to present in the genus *Swertia* in the literature [9], the structure of the active crystal from *Swertia purpurescens* was elucidated as follow. According to ¹H-NMR spectrometer (600 MHz), only one aromatic methoxy proton $\delta = 3.9079$ ppm was detected in the active crystal of *Swertia purpurescens* so that structure I and II were possible structures. Among the two possible structures I and II, I was confirmed by Nuclear Overhauser Effect (NOE). Structure II was not revealed by NOE due to lack of one proton adjacent to methoxy groups (Fig. 3).

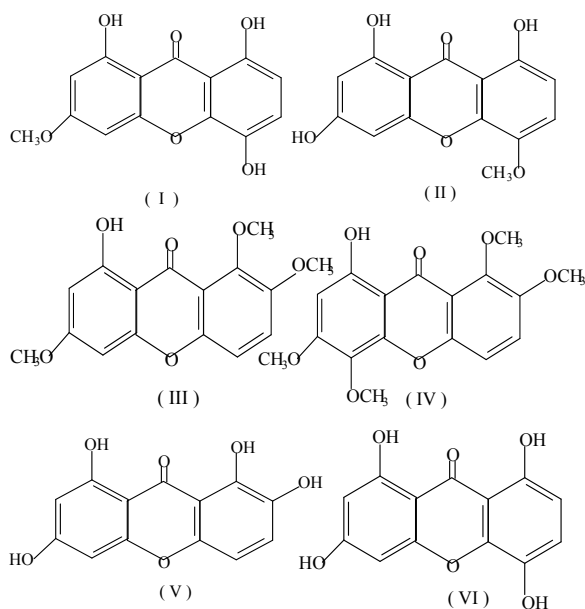


Fig. 3. Six possible xanthone structures of *Swertia purpurescens*

In this experiment, irradiation of methoxy methyl protons ($\delta = 3.9079$ ppm) with low radio frequency responses the two aromatic

protons ($\delta = 6.663$ ppm) and ($\delta = 6.42$ ppm) respectively, and the coupling constant of both of these aromatic protons ($J = 1.8$ Hz) as meta to each other (Fig. 4).

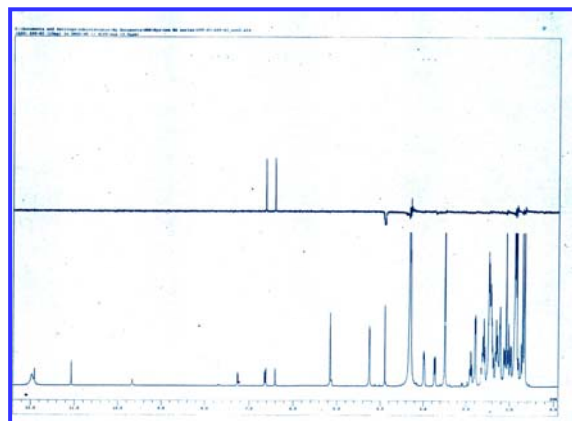


Fig. 4. Nuclear over hauser effect structure

It gave rise to the evidence that only structure I, Bellidifolin (1, 5, 8-trihydroxy-3-methoxy xanthone), was determined for the chemical configuration of active crystal of *Swertia purpurescens* in this study. NOE spectrum is shown in Fig. 4.

DISCUSSION

Although a single local name, Pan Kha was known by the local people of Kayah State, this study could reveal three species of genus *Swertia* as *S. affinis*, *S. angustifolia* and *S. purpurescens*. Since there is no report on Myanmar resource of *Swertia* species for the botanical identity before this study, the clarification of botanical species of Pan Kha is the first report that *Swertia* as *S. affinis*, *S. angustifolia* and *S. purpurescens* are available in the Kayah State of Myanmar. On the use of these plants by local people for health purpose, it could be reported that these plants were not acute toxic at the dose of 2.8g/kg/day (ethanol extract and H₂O) and 2-4 g/kg/day (CHCl₃) in this study. Regarding the antimalarial activity, some extracts of each plant were observed to be active at certain extent, of which ethanol extract of *S. purpurescens* was reported to be the most active as the

findings in *in vivo* experiment model. Serial chemical separation of ethanol extracts of *S. purpurescens* by TLC and column chromatographic techniques provided a significant amount of a crystalline substance together with minor constituents. The promising activity of this crystalline substance was interestingly found when it was retested in both of *in vivo* and *in vitro* models. Therefore, this study could have revealed the antimalarial active principle of *S. purpurescens*. Finally, the antimalarial active crystalline compound could have been identified as Bellidifolin by means of advanced analytical instruments such as FTIR, ¹HNMR and NOE spectronic techniques. According to the literature available which had reviewed the xanthone and antimalarial activity [7, 10], synthetic xanthone compounds and some natural xanthones from *Swertia* species (not from *S. purpurescens*) were reported to possess antimalarial activity. However, *S. purpurescens* and its chemical compound, Bellidifolin, were not seen for their antimalarial activity in the available previous reports. Therefore, this study could structurally elucidate an active compound, Bellidifolin, from *S. purpurescens* and for the first time, the possessing of antimalarial activity in *S. purpurescens* could be reported.

REFERENCES

1. Hakin MA. Physicochemical Standards of Unani formulations, Ministry of Health and Family Welfare, New Delhi, India, 1987; 244.
2. Litchfield JT & Jr. Ardr Wilcoxon FA. Simplified method of evaluating dose effect experiments, *Journal of pharmacological, experimental therapeutic* 1949; 96: 99, 33.
3. Peters W. Competitive relationship between eperythrozoon coccidia and *P. berghei* in the mouse. *Experimental parasitology* 1965;16; 158.
4. Myint Lwin. Interaction of chemotherapy and the immune response in experimental malaria infections, *Ph.D. Thesis*, University of London, London School of Hygiene and Tropical Medicine, 1978.
5. Riechmann KH, *et al.* Drug sensitivity of *Plasmodium falciparum* *in vitro* microtechnique, *Lancet* 1978; I; 22, 23.
6. Harbone JB. Phytochemical Methods, Chapman and Hall, London, 1984.
7. Rastogi RP & Metrotra BN. Compendium of Indian Medicinal Plants, New Delhi, 1994; 5: 815-816.
8. Saboor A, Mohammad I & Isar K. Phytochemistry, Melbourne, Australia, 1873; 12: 2542-2543.
9. Ghosal S, Sharma PV. & Chaudhuri RK. Bhattacharyask. *Journal of Pharmacology* 1975; 1: 80-83.
10. Struwe L & Albert V. Eds. Gentianaceae Systematics and Natural History, Cambridge University press, 2002: 574-612.

Epidemiological study of snakebites cases admitted to two township hospitals of Ayeyawady Division

Aye Aye Myint, **Tun Pe & *Swe Win*

**Immunology Research Division*

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

****Department of Health*

Snakebite is endemic in rice growing divisions of Myanmar including Ayeyawady Division. The average case fatality rate (CFR) of poisonous snakebite of the whole country is 7.43%. Ayeyawady Division has the highest case fatality rate (CFR) in the country. In order to determine the morbidity, mortality and the causes of high CFR of snakebite in Ayeyawady Division, a retrospective analysis of case notes of snakebite victims admitted to Mawlamyainggyun and Einme hospitals of Ayeyawady Division for the last 6 years (2001-2006) was carried out. Total number of snakebite cases in Mawlamyainggyun was 129 and that of Einme 48. Majority of the victims were male and bitten on lower limbs. Russell's viper bite accounted for 33.3% and cobra 10-17%. Majority (79-87.5%) of Russell's viper bite cases had systemic envenoming at admission and 44-75% of them had systemic complications. The CFR of poisonous snakebites of Mawlamyainggyun was 26.2% (17/65) and that of Einme 45.45% (10/22). The CFR of Russell's viper bite was 66% if the victims arrived hospitals within 6 hr after the bites and it increases with delay in arrival time. A long hour of travel [9.30 hrs (Mawlamyainggyun) and 8.29 hrs (Einme) (median)] to get to the hospitals leading to development of systemic envenoming and complications were contributing factors in causing high CFR. Since other contributing factors like use of protective wears and effective first aid measure, prehospital antivenom and means of transportation of the victims were not available in the record, a prospective study of snakebite cases admitted to township hospitals is needed to answer the pressing questions.

INTRODUCTION

Snakebite is endemic in rice growing divisions of Myanmar which includes Ayeyawady Division. It has a moderate snakebite morbidity 889 bites/year (ranged 744-995 bites/year) and has the highest case fatality rate (CFR) 17.75% of the country. Ninety-two percent of (24/26) townships of Ayeyawady Division have the case fatality rate ranging from (11-40%) throughout past 8 years (1998-2005) [1]. The high CFR of snakebite in Ayeyawady Division raises a concern among health care providers and the possible factors contributing to it need to be sought. This study aimed to determine the morbidity and CFR of two

townships (Einme and Mawlamyainggyun) of Ayeyawady Division and possible causes contributing towards the high CFR.

MATERIALS AND METHODS

Two townships, Einme and Mawlamyainggyun of Ayeyawady Division, which have the CFR of 40.5% and 26.2% respectively identified from an earlier epidemiological study of poisonous snakebite cases of Myanmar [1] were selected for the study. The case notes of snakebite cases admitted to the respective hospitals for the period of 2001-2006 were requested from respective hospitals through the divisional health authority. Retrospective analysis of data

from the case notes of snakebite cases were coded, entered and analysed in Epi Info version 6.04d.

RESULTS

Yearly incidence, CFR and trend of poisonous and total snakebites of two townships are shown in the Table 1 & 2. Data from earlier epidemiological study of poisonous snakebites from the Department of Health Planning [1] were included for comparison.

Table 1. Comparison of two data sources for case fatality rate among snakebite cases admitted to Mawlamyainggyun Hospital from (2001-2006)

Year	DHP		Current study	
	PSB		PSB	
	CFR (%)		CFR (%)	
2001	1/10	10	7/13	53.8
2002	0/32		0/5	
2003	6/10	60	7/1	14.2
2004	9/18	50	3/17	17.6
2005	17/29	58.6	4/14	28.6
2006	3/29	10.3	2/9	22.2

DHP= Department of Health Planning

PSB = Poisonous Snake Bite

CFR = Case Fatality Rate

Table 2. Comparison of two data sources for case fatality rate among snakebite cases admitted to Einme Hospital from (2002-2006)

Year	DHP		Current study	
	PSB		PSB	
	CFR (%)		CFR (%)	
2002	0/6		1/2	50
2003	4/11	36.4	1/3	33.3
2004	3/6	50	4/7	57
2005			1/4	25
2006	1/19	5.26	3/6	50

Demography

Majority of snakebite occurred in age groups from 11 to 50 year, accounting for 83.5% (n=41) in Einme, and 86% (n=129) in Mawlamyainggyun, 7% under 10 years and between 51-80 years in both townships.

Clinical features

In Mawlamyainggyun, 79% (34/43) of Russell's viper bite presented with systemic

envenoming on admission of which 44.2% (19/34) had systemic complications, such as shock (n=5), renal failure (n=4), haematuria (n=1), haematemesis (n=1), haemoptysis (n=1), haematuria and malena (n=1), renal failure with haematemesis and malena (n=2), shock with renal failure (n=1), shock with haematemesis (n=1) or haematuria (n=1) or haemoptysis (n=1). In Einme, 87.5% (14/16) of Russell's viper bite cases had systemic envenoming on admission of which 75% (12/16) developed systemic complications such as renal failure (n=6), shock (n=2), renal failure with shock (n=1) and haemorrhagic manifestations (n=2). Cobra bite cases presented with ptosis, blurring of vision and dysarthria.

Species of snake

Russell's viper bite accounted for 33.3% (43/129) in Mawlamyainggyun and 33.3% (16/48) in Einme, cobra bite 17% (22/129) in Mawlamyainggyun, and 10.4% (5/48) in Einme, Green snake 2% (1/48) in Einme, unknown bites 49.6% (64/129) in Mawlamyainggyun and 54.1% (26/48) in Einme.

Time bite and admission

Median time taken for snake bite patients to get to Einme Hospital was 5.82 hrs (0.15-72 hrs) (n=27) and that of poisonous snake bites 8.29 hrs (1.05-72 hrs) (n=12). Majority 78.6% (22/28) of the victims reached the hospital within 6 hrs after the bite, 10.7% (3/28) between 7-12 hrs, 7.14% (2/28) between 13-24 hrs and 3.5% (1/28) between 30-72 hrs after the bite. In Mawlamyainggyun, it took 8.9 hrs (15 min-72 hrs) (n=75) for the victims to reach hospital and 9.3 hr (0.30-72 hrs) (n= 48) for poisonous snake bites. The majority 62.6% (47/75) of the victims arrived at the hospital within 6 hr after the bite, 17.3% (13/75) between 7-12 hrs, 13.3% (10/75) between 13-34 hrs and 6.6% (5/75) between 30-72 hrs after the bite. Due to incomplete case records, all data could not be retrieved.

Clinical outcome

In Mawlamyainggyun, six (4 Russell's viper bites and 2 cobra bites) died at the hospital

while undergoing treatment. Majority 66.6% (4/6) Russell's viper bite admitted to the hospital within 6 hrs after the bite were fatal, 75% (3/4) in between 7-12 hrs, 75% (3/4) in between 13-24 hrs, 100% (2/2) at 72 hrs after the bite. Six systemic envenomed Russell's viper bite cases with complications, such as, shock, who did not seek further treatment at the hospital were believed to be fatal. Four systemic envenomed Russell's viper bite cases with complication, renal failure or shock were referred to Yangon General Hospital and their outcomes were not known. For cobra bite, 2/4 cases admitted to hospital within 6 hrs after the bite died, none in between 7-12 hrs (n=4) and 13-24 hrs (n=2) after the bite. Two cobra bite cases, one admitted on 4.45 hrs after the bite given 40 ml antivenom died on admission and another 3 hrs after the bite, given 30 ml antivenom died 3 hrs after admission.

In Einme, two cobra bite cases admitted to hospital, within 2 hrs after the bite in one and biting time not recorded in another, given 20 ml of cobra antivenom died at hospital following respiratory arrest. No deaths due to Russell's viper bites were on the record. Eight systemic envenomed Russell's viper bite cases with complications: shock or renal failure victims who did not seek further treatment at the hospital were assumed to be fatal. They were 4/6 Russell's viper bites admitted to hospital within 6 hrs after the bite, 2/2 in between 13 to 19 hrs, and 1/1, in 72 hrs after the bite. No record on time of admission was noted in one case.

Antivenom usage in Russell's viper bites

In Mawlamyainggyun, data on antivenom usage in 42/129 Russell's viper bite were available for analysis. 10 ml (n=20), 20ml (n=5), 30 ml (n=2), 40 ml (n=22), 60 ml (n=1) 80 ml (n=8), 110ml (n=1) and 140 ml (n=1). In Einme, information on the usage of antivenom was only available for 14/48 Russell's viper bite cases: 20 ml (n=1), 40 ml (n=4), 80 ml (n=7), 100 ml (n=1) and 200 ml (n=1).

Antivenom usage in cobra bite

In Mawlamyainggyun: cobra antivenom was given at variable doses: 20 ml (n=4), 30 ml (n=4), 40 ml (n=12), 50 ml (n=1) and 80 ml (40+40 ml) in one. In Einme, cobra bites were given 20 ml (n=2) and 40 ml antivenom (n=2) and none in one.

Antivenom usage in unknown bites

Among unknown bites, cobra antivenom was given to four cases, 3 suspected cobra bites; 10 ml in one, and 40 ml in two and 40+40 ml in one suspected krait bite in Mawlamyainggyun. However, only one unknown bite was given Russell's viper antivenom (40 ml) in Einme, otherwise the majority of unknown bites were not given antivenom.

DISCUSSION

The study indicated that the morbidity of snakebite in two study townships was not high but the case fatality rates were. The trend of snakebite observed in this study is in agreement with the report from the Department of Health Planning [1]. However, there is a discrepancy in numbers of bite and fatal cases. The demographic data of the snakebite victims are the same as that observed in snakebite cases in other townships [2]. The majority of the victims were male, age ranged between 11-50 years and bitten on lower limbs. The incidence of Russell's viper bite in two townships was low (33%) and that of cobra bite was high (10-17%) compared to the incidence of Russell's viper (60%) and cobra 6% in study of snakebites in six township hospitals [2]. It was likely to be that the pattern of snakebite reflects the occupation of the local residents.

Time taken to transport the victims from site of bite to hospital was twice longer than that of snakebite cases taken in other townships of Ayeyawady: Yaekyi (4.55 hrs) and Danuphyu (4.45 hrs). Long hours of time taken to transport the victims in the absence of effective first aid contributed to high

incidence of systemic envenoming (79 and 87%) compared to Yaekyi (76%) and Danuphyu (58%) (average 43%) and development of systemic complications (44-75%) compared to Yaegyi 54% and Danuphyu 67% (average 42%) [2].

Antivenom usage

Antivenom usage in Russell's viper and cobra bites in the two townships was in agreement with the guidelines [3]: majority of Russell's viper bites were given 40 ml and 80 ml with the exception of four, given 100 ml and 200 ml in Einme and 110 ml and 140 ml in Mawlamyainggyun. A wide dose ranged from 10 ml to 80 ml were given in cobra bite cases in Mawlamyainggyun, however, the majority were given 40 ml antivenom. Majority of unknown bites were not given antivenom except a few cases. Since antivenom is expensive to produce the use of large dose of antivenom should be justified. It is emphasized that specific antivenom should be given to specific bite and cobra antivenom was not helpful in treating other neurotoxic bites [4] like suspected krait bite given 40+40 ml of cobra antivenom.

It is highly likely that contributing causes of high case fatality rate of Russell's viper bites in the two townships were long hours of time taken to transport the victims to the hospitals and development of systemic envenoming and complications on arrival at the hospitals. Moreover, CFR increases with delay in arrival time. Because of incomplete documentation, other contributing factors could not be retrieved from the hospital records. It is suggested that Medical Officers looking after the snakebite victims should be trained to keep full documentation of patients for references and it is their responsibility to upgrade and maintain their medical skills.

It is emphasized that use of correct effective first aid [5], administration of prehospital antivenom [6], avoid wasting valuable time after the bite and correct and speedy transportation of the victim to the health

center will delay on set of envenoming and help in bringing down the case fatality rate.

It is speculated that timely introduction of assisted ventilatory support to the fatal cobra bites in the hospital could have saved the lives of the victims since reversion of neurotoxic symptoms after antivenom takes time. It has been documented that many lives of neurotoxic envenomed victims have been saved by artificial ventilation even in the absence of specific antivenom [7]. Many lives of cobra bite victims who developed respiratory arrest on the way to hospitals could have been saved by artificial ventilation which could be provided by the relatives/health workers who accompanied the victim to the hospital (personal communication).

It is suggested that Medical Officers looking after the snakebite victims in township hospitals should be trained to insert endotracheal tube [8] and provide mechanical ventilation to the neurotoxic envenomed victims who develop respiratory difficulty or arrest. Moreover, this valuable life saving message of providing firstaid to neurotoxic envenomed victims who develops respiratory arrest on the way to hospital should be promoted among health-care providers and in community.

It is surprising to learn that there were no records of death due to Russell's viper bite in past 6 years in Einme hospitals. Since there is no cold storage facility in the district hospitals, patients in terminal illness care usually taken home and mortality rate of disease in district hospital will be grossly underestimated. However, in our analysis of the cases, all the terminal cases were regarded as fatal cases.

In conclusion, high case fatality rate in the two study townships was documented. Long hours of transportation of patients to hospital plus development of systemic envenoming and complications at admission are contributing causes for high case fatality rate. Because of incomplete documentation, a well designed prospective hospital based

study of snake bite will provide more information on answering the pressing questions.

REFERENCES

1. Aye Aye Myint, Tin Lin Myint & Tun Pe. Poisonous snakebite of Myanmar with special references to the bite and case fatality rate (1998-2005). *Myanmar Health Sciences Research Journal* 2007; 19(2): 87-93.
2. Tun Pe, Aye Aye Myint, Sann Mya, Khin Aye Kyu, Kyaw Than *et al.* Clinical features of Russell's viper (*Daboia russelii siamensis*) bite cases admitted to six township hospitals of snakebite endemic divisions of Myanmar. In: *Management of snakebite and research* 2002; 34-40.
3. Snake bite Research group. *A guide to management of snake bite. DMR (LM) 2004.*
4. Tun Pe, Tin Myint, Aung Htut, Than Htut, Aye Aye Myint & Nu Nu Aung. Envenoming by Chinese krait (*Bungarus multicinctus*) and banded krait (*B. fasciatus*) in Myanmar. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1997; 91: 686-688.
5. Tun Pe, Sann Mya, Aye Aye Myint, Nu Nu Aung, Khin Aye Kyu & Tin Oo. Field trial of efficacy of local compression immobilization first aid technique in Russell's viper (*Daboia russelii siamensis*) bite patients. *Southeast Asian Journal of Tropical Medicine and Public Health* 2000; 31: 346-348.
6. Tun Pe, Aye Aye Myint, Khin Aye Kyu & Sann Mya. Evaluation of prehospital antivenom in management and outcome of Russell's viper (*Daboia russelii siamensis*) bite cases admitted to Taungdwingyi hospital. *Myanmar Health Sciences Research Journal* 2003; 15 (1-3):1-5.
7. Tin Myint & Tun Pe. Forty – five days mechanical ventilation in Chinese krait (*Bungarus multi-cinctus*) bite. *Myanmar Health Sciences Research Journal* 2002; 14(1-3): 45-47.
8. Warrell DA. The clinical management of snakebites in the Southeast Asian region. *Sout-Eeast Asian Journal of Tropical Medicine and Public Health* 1991; 30: Supplement 1.

**Detection of alpha-thalassaemia in newborn cord blood
from North Okkalapa General Hospital**

Ne Win, **Khine Khine Phyu, *Myat Mon Oo, ***Mya Thandar Win,
***Kyaw Soe & ***Minn Minn Myint Thu*

**National Health Laboratory, Department of Health*

***Department of Zoology, University of Yangon*

****Pathology Research Division*

Department of Medical Research (Lower Myanmar)

Thalassaemia is a heterogeneous group of disorders with a genetically determined reduction in the rate of synthesis of one or more types of normal haemoglobin polypeptide chain. A total of 308 cord blood samples from North Okkalapa General Hospital were screened for the presence of thalassaemia and haemoglobinopathies. The tests included haemoglobin electrophoresis, Hb A₂ estimation, and H inclusion bodies examination. Hb F alone band was found in 256 cases (83.12%), Hb EF band revealed in 25 cases (8.12%), Hb FH band was seen in 23 cases (7.47%) and Hb EFH band revealed in 4 cases (1.3%). Haemoglobin H was seen in 27 cases (8.77%) in Hb FH and Hb EFH bands. Among them, 21 cases had Hb H inclusion percent < 10% of RBC and 3 cases had Hb H inclusion percent > 30%. Twenty-four cases showed Hb A₂ < 2%. According to this study, on cord blood electrophoresis, α -thalassaemia prevalence is comparable to those previously reported (10%).

INTRODUCTION

The thalassaemias, the commonest monogenic diseases, are a family of inherited disorders of haemoglobin (Hb) synthesis characterized by a reduced output of one or more of the globin chains of adult Hb. Most of the important forms of thalassaemias are inherited in a Mendelian fashion; carrier parents who are symptomless have a one in four chance of having a severely affected child [1].

The thalassaemias are distributed across the Mediterranean region, the Middle East, the Indian subcontinent, and throughout South East Asia, including Myanmar. In many of these countries gene frequencies for the different thalassaemias and structural Hb variants are high. As social conditions improve in developing countries and childhood mortality due to infection and malnutrition declines, children with thala-

ssaemia who would previously have died early in life are now surviving long enough to require treatment. The reason for very high frequency of thalassaemia is that carriers are protected from the consequences of infection with malaria parasite [1].

Thalassaemias are generally classified according to the particular globin chain that is ineffectively produced, as two main groups, α and β . The α -thalassaemias are disorders in which there is defective synthesis of α chains, i.e. Hb A, Hb A₂ and Hb F. The deficiency of α chains leads to an excess of γ chains in the fetus and of β chains in the adult [2].

Cord blood is composed of Hb A 20-25% and Hb F 70-90% of total Hbs [3]. In α -thalassaemia, α chains are shared by fetal and adult Hb; and hence the disease is manifest in both fetal and in adult life. Furthermore, excess γ and β chains do not precipitate immediately in the bone marrow

like α chain but produce the physiologically useless and unstable tetramers: γ_4 (Hb Bart's) and β_4 (Hb H). Since the α genes are duplicated, the genetics of thalassaemia is more complicated than that of β -thalassaemia. The genetic makeup of normal individuals can be written as $\alpha\alpha/\alpha\alpha$. Loss of both α genes on a chromosome is called α^0 -thalassaemia, and is represented $-\alpha/\alpha$. Loss of one of the linked pairs of a globin genes is called α^+ -thalassaemia, $-\alpha/\alpha$. The homozygous state for α^0 -thalassaemia produces intrauterine death with a profoundly anaemic and hydropic fetus: the Hb Bart's hydrops fetalis syndrome. Compound heterozygotes for α^0 - and α^+ -thalassaemia have a milder illness characterized by anaemia and splenomegaly which is called Hb H disease. Carriers for α^0 -thalassaemia and homozygotes for α -thalassaemia have mild hypochromic anaemia, while carriers for α^+ -thalassaemia have no haematological abnormalities [1].

The carrier state for all the important thalassaemias can be identified and methods for their prenatal diagnosis are well established. The first brief report of Hb H disease in Myanmar was published by Aung Than Ba Tu *et al.* in 1968 [4]. Their observations indicated that both α - and β -thalassaemias are prevalent in Myanmar. Hb Bart's in the cord blood for the detection of the incidence of α -thalassaemia was reported [5].

Haemoglobin H inclusion bodies (precipitated beta chain tetramers) are found in α -thalassaemia. Haemoglobin H has red cells which on exposure to brilliant cresyl blue developed multiple blue green spherical inclusions like the pitted pattern on a golf ball. Sometimes, haemoglobin H inclusion is not demonstrated. Definitive diagnosis can only be given by precise methods in molecular biology [6].

In Myanmar, there is high incidence of the important thalassaemias; i.e. α -thalassaemia 10%, Hb E 28%, β -thalassaemia 0.54-3.8% and 1-4.9 births per 1,000 infants with a

major haemoglobinopathy [7]. Therefore, α -thalassaemia is an important health problem for Myanmar and it is essential to detect and identify the carrier state of thalassaemia and abnormal haemoglobin. Therefore, the present study is undertaken with the aims:

1. To detect the α -thalassaemia in newborn babies
2. To determine the haemoglobin bands among newborn cord blood
3. To estimate the content of haemoglobin A₂
4. To identify the haemoglobin H inclusion bodies

MATERIALS AND METHODS

A cross-sectional study was conducted to detect alpha-thalassaemia in newborn cord blood from North Okkalapa General Hospital during 2004-2006. A total of 308 newborn babies were included in this study. After getting an informed consent from the mother, thorough history taking was done. Diagnosis of α -thalassaemia trait was based on detection of haemoglobin H inclusion body and confirmed by finding Hb A₂ concentration less than 2% of total haemoglobin concentration (HbA₂ concentration 2-4% is normal) [6].

Haemoglobin H inclusion

For Hb H inclusion, equal volumes of fresh blood or blood collected into EDTA and 10 g/l brilliant cresyl blue (BCB) were mixed together in a small test tube. The preparation was kept at 37 °C for 20 min, and films made at intervals during this time. The films were allowed to dry and examined without counterstaining. Hb H precipitates as multiple pale-staining greenish blue, almost spherical bodies of varying size which can be clearly differentiated from the darker staining reticulo-filamentous material of reticulocytes. The number of cells containing inclusions varies according to the type of α -thalassaemia.

Haemoglobin A₂ determination

Hb A₂ was determined by cellulose acetate electrophoresis followed by elution method. The cellulose acetate strips were soaked in the buffer containing tris (hydroxymethyl) aminomethane, ethylenediaminetetraacetic acid, boric acid (TEB buffer, pH 8.9) for 5-10 min and gently blotted dry. They were then stretched across the bridge of the electrophoresis tank connected to the TEB buffer chamber by a double-layered filter paper with 10µl of hemolysated blood (Hb concentration 8-10 g/dl) applied via a microcapillary pipette and allowed to soak in.

Electrophoresis was carried out at 220 V (approx: 5mA) for about 1½ hours, after which the strips were removed and the Hb A and Hb A₂ zones were cut into small strips and eluted in 15 ml and 1.5 ml TEB buffer respectively. When elution was complete (approx: 30 mins) the tubes were inverted to mix the contents, and the optical density (OD) was determined at 415 nm. The percentage Hb A₂ was then calculated as:

$$\%HbA_2 = \frac{OD^{415} HbA_2}{OD^{415} HbA_2 + (OD^{415} HbA \times 10)} \times 100$$

Iso-Electric Focusing (IEF)

Typical 1mm polyacrylamide gel was prepared with following gel composition.

Distilled Water	3 ml
Acrylamide	3 ml
Sucrose	11 ml
Pharmolyte	300 µl (pH 6.7 – 7.7)
Ampholine	300 µl (pH 7.0 - 9.0)
Ampholine	150 µl (pH 3.5 -10.0)
Ammonium Persulfate	20 µl
NNN'N' tetramethylethylenediamine	50 µl

Ten microliter of whole blood was mixed with 50 µl of saponin in microtitre plate wells. The three filter paper strips were placed accordingly (1 in centre and 2 in periphery of the gel plates). The central strip was soaked homogeneously with 0.2 mole of phosphoric acid solution and the peripheral strips were soaked homogeneously with 0.2 mole of NaOH solution.

Prefocusing was done for 20–30 minutes with 300 V.

Pieces of filter papers (5 mm length) were placed into each well of samples and then soaked with blood. The soaked paper was placed on the gel plates. Then again, continued focusing was done with 400 V for 2 hours. The separation was carried out at a constant pH [6].

RESULTS

Detection of haemoglobin H inclusion bodies

Out of total 308 cases, Hb H inclusions appeared as golf balls were detected in 27 cases (8.77%) of which 18 were males and 9 were females. Among 27 cases (8.77%), 21 cases (6.82%) showed H inclusion bodies less than 10% and they were suggested as α-thalassaemia trait. Three cases (0.97%) showed H inclusion bodies 25-30% and they were diagnosed as thalassaemia intermedia. The remaining 3 cases (0.97%) showed H inclusion bodies more than 30% and were Hb H disease (Table 1).

Table 1. H inclusion bodies in newborn cord blood

	H negative (normal)	Positive(H)			Total
		H<10% α-thalassaemia trait	H 25-30% α-thalassaemia intermedia	H>30% Hb H disease	
Male	130	9	3	3	145
Female	151	12	0	0	163
Total	281	21	3	3	308

H = Haemoglobin H inclusion bodies
H<10% = H less than 10% of red blood cell
H 25-30% = H 25-30% of red blood cell
H>30% = H more than 30% of red blood cell

Iso-electric focusing (IEF)

Hb F alone was seen in 256 cases (83.12%) of which 120 were males and 136 were females. Hb EF band was revealed in 25 cases (8.12%) i.e. 9 males and 16 females and Hb FH was seen in 23 cases (7.47%) i.e. 12 males and 11 females. Hb EFH band was detected in 4 cases (1.3%) i.e. 3 males and 1 female (Fig 1 & Table 2).

Table 2. Various types of haemoglobin bands detected by iso-electric focusing using polyacrylamide gel in newborn cord blood

	F alone	EF	FH	EFH	Total
Male	120	9	12	3	144
Female	136	16	11	1	164
Total	256	25	23	4	308

F = haemoglobin F band

EF = haemoglobin E and F bands

FH = haemoglobin F and H bands

EFH = haemoglobin E, F and H bands

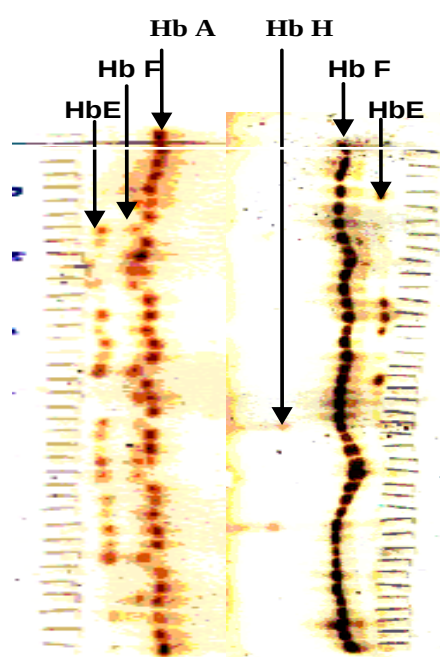


Fig. 1. Haemoglobin bands detected by iso-electric focusing

Table 3. Haemoglobin A₂ level in newborn cord blood

	Hb A ₂ <2%	Hb A ₂ 2-4%	Hb A ₂ 4-9%	Hb A ₂ >9%	Total
Male	12	122	2	9	145
Female	12	143	0	8	163
Total	24	265	2	17	308

Haemoglobin A₂ level

In 308 cases, normal Hb A₂ level (2-4%) was seen in 265 cases (86.04%) of which 122 were males and 143 females. Hb A₂ less than 2% was seen in 24 cases (7.79%)

where 12 males and 12 females were found. Hb A₂ level 4-9% was detected in 2 cases (0.65%) both of which were males. Hb A₂ more than 9% was seen in 17 cases (5.52%) i.e. 9 males and 8 females (Table 3).

DISCUSSION

There are about 55 million people in South-East Asia carrying a thalassaemia gene or a Hb E gene. Control and prevention programs at any level are essential since this disease burden is expanding and at large. It has been predicted and speculated that thalassaemias/haemoglobinopathy are likely to impose an increasing health problem for many developing countries during the early part of the new millennium [8].

In this study, we demonstrated the prevalence of α -thalassaemia by screening of 308 samples of newborn cord blood by haemoglobin electrophoresis using iso-electric focusing by polyacrylamide gel, brilliant cresyl blue for examination of H inclusion bodies and cellulose acetate membrane for quantitative estimations of Hb A₂.

In α -thalassaemia, red cell haemoglobin H inclusion (H positive) (golf-ball shaped) are demonstrated. Haemoglobin H disease (H inclusion bodies 30% and above) has high incidence in the South-East Asia and occurs sporadically in the Middle East [9]. H inclusion bodies less than 10% are α -thalassaemia trait, H inclusion positive 25-30% is α -thalassaemia intermedia and H inclusion bodies more than 30% is haemoglobin H disease [6]. In 1956, the first report of haemoglobin H disease in Myanmar was made by Aung Than Batu and colleagues. They indicated a high prevalence of Hb H disease [5].

In this study, we found that H inclusion positivity was seen in 8.77% and H inclusion was negative in 91.23%. Among H inclusion positive cases, 21 cases (6.82%) showed H inclusion less than 10%, three cases (0.97%) showed H inclusion 25-30%

and three cases (0.97%) showed H inclusion more than 30%. Hb H and Hb Bart's were associated with a severe form of α -thalassaemia, Hb Bart's is γ_4 and Hb H is β_4 . The cases with Hb Bart's may die either in utero or soon after birth [10]. Taking into consideration of hemoglobin Bart's hydrops and Hb H in the severe form of a thalassaemia, probability of risk couples must be definitely increased. If a mother is a carrier for α^0 -thalassaemia her pregnancy is at risk for the Bart's hydrops fetalis syndrome, whereas the worst possible outcome of a pregnancy involving a woman homozygous for α^+ -thalassaemia is the much milder condition, Hb H disease.

Weatherall (1998) has been suggested that over the next decade, it will be essential to make the thalassaemia problem more visible to governments and international health agencies that are involved in health care in the developing countries. This will require detail population surveys to determine the gene frequencies of the important forms of thalassaemia, together with a better understanding of their natural history and of the factors that modify their clinical phenotypes [11].

Haemoglobin H disease (β_4) indicates that the α -thalassaemia gene would be present in these patients who form Hb H inclusions in red cells on exposure to brilliant cresyl blue staining test. In normal adult blood, haemoglobin A consists of more than 90% of total haemoglobin and haemoglobin F is less than 1% and the remaining portions consist of haemoglobin A₂ 2.5-4%.

In the present study, haemoglobin F was present alone or combined with other bands in newborn cord blood. It is normal finding for newborn babies. In normal newborn cord blood, haemoglobin F accounts for 70-90% of total haemoglobin [3].

Aung Than Batu *et al.* reported that approximately 10% of Burmese cord blood had Hb Bart's indicating a high prevalence of alpha thalassaemia trait and possibility of Hb H being frequent [5]. Therefore,

according to this study, on cord blood electrophoresis, α -thalassaemia prevalence is comparable to those previously reported.

Health education and genetic counseling are the important initial steps of effective prevention program of thalassaemia which should be developed in Myanmar in the near future. Standards for appropriate Hb testing by various electrophoresis techniques must be set up, Hb A₂ estimation should be encouraged as a routine screening test. These options should be developed before considering prenatal diagnosis. We suggest that intensive community education is of prime importance which should precede the institution of genetic screening programmes. At least family planning and birth spacing practice after health education and genetic counseling may hopefully reduce the rate of birth of an infant with thalassaemia/ haemoglobinopathy.

This study highlights the fact that screening and genetic counseling, can support prevention and control programs for thalassaemia. Systemic prevention and control program should be planned by the cooperation of both the public and private sectors.

ACKNOWLEDGEMENT

We thank Professor Dr. Khin Saw Hla, (Retired Head), Department of Obstetric and Gynaecology, North Okklapa General Hospital for her permission to collect the cordblood samples.

REFERENCES

1. DJ Weatherall. The Thalassaemias. *British Medical Journal* 1997; 314: 1675-1677.
2. De Gruchy. Disorders of haemoglobin structure and synthesis. In: *Clinical Haematology in Medical Practice*. Firkin F *et al.* (eds). Blackwell Scientific Publication Oxford London Edinburgh 1989:137-171.
3. Weatherall DJ & Clegg JB. The thalassaemia syndromes. In: *Historical Perspectives*, 3rd ed. Blackwell Scientific Publications, Oxford UK. 1982: 147.

4. Aung Than Batu, Hla Pe, Nyunt Mg & Khin Kyi Nyunt. The thalassaemia in Burma, Union of Burma. *Journal of life Sciences* 1968; 241-247.
5. Aung Than Batu, Hla Pe & Khin Kyi Nyunt. Haemoglobinopathies in Burma II Haemoglobin H disease. *Tropical and Geographical Medicine*. 1971; 19-25.
6. Brozovic M & Henthorn J. Investigation of abnormal haemoglobin and thalassaemia. In: *Practical Haematology*: Dacie JV.& Lewis SM. (eds) 8th ed. Churchill Livingstone, Edinburg. 1995; 249-286.
7. Ne Win, Thein Myint Thu, Phyu Phyu Aung *et al*. Antenatal screening of beta thalassaemia and haemoglobin E from the perspectives of detection of couples at risk for a newborn with severe thalassaemia. *Journal of Myanmar Military Medicine* 2003; 009: 25-28.
8. Wong HB. Prevention of thalassaemia in South East Asia. *Annals of Academic Medicine Singapore* 1985; 14 (4): 654-65.
9. Minnich, V, , Nakorn SN, Tuchinda S, Wasi P& Moore CV. Inclusion body anaemia in Thailand: Haemoglobin H disease. Proceedings of the 6th International Society of Haematologis, Boston, Grune and Stratton, New York 1958.
10. Hunt JA & Lehmann H. Abnormal human haemoglobins, haemoglobin Bart's: A foetal haemoglobin without α chains. *Nature* 1959: 872.
11. Weatherall DJ. Thalassaemia in the next millenium. Key note address. *Ann-N Acad-Sci*. 1998; 30: 8501-8509.

Family and community practices of newborn care in Pyay District

**Kyu Kyu Than, *ThanTun Sein, *Ko Ko Zaw, *Mon Mon, *Myo Myo Mon,
**Thein Aung, **Khin Mar Aye & **Myint Lwin*

**Department of Medical Research (Lower Myanmar)*

***Department of Health*

The study aims to assess the level of and barriers to key family and community practices concerning newborn care in Pyay District. The study was done from January to June 2005 in five townships of Pyay District. A total of 1090 mothers with under five years old children were interviewed using a pre-tested face-to-face interview questionnaire. For the qualitative assessment, a total of 20 Focus Group Discussions were carried out with 157 mothers. In-depth Interviews were also carried out with 15 health care providers of child care and 7 mothers with neonates. Observations were also done on some of the family and community practices in relation to newborn care. Eighty percent of the mothers in the study had home deliveries. Sixty-two percent of the mothers were delivered by a trained birth attendant. Although most of the deliveries used clean sterilized instruments, there is still practice of using un-sterilized bamboo slab for cutting the umbilical cord by traditional birth attendants. Fifty-four percent of the babies in the study were immediately bathed after birth. Nearly seventy percent of the babies in the study were also wrapped with clothes from head to toe immediately after delivery. All the newborn children in the study were breastfed immediately after birth. But only one third of the breastfed children received exclusive breastfeeding. The reasons underlying the key family and community practices concerning newborn care were also explored. The study highlights that newborn care from a family and community perspective is an important input for the programmes.

INTRODUCTION

Children are highly vulnerable to poor health outcomes and fatality for various reasons and child health development plays the integral and vital role in improving the health status of populations. More than 10 million children die each year, most from preventable causes [1]. Among them, neonates bear the brunt of under five years mortality. In 2003, 73% of under-five deaths occurred during infancy and 34% of infant deaths occurred during the neonatal period [2]. Almost all neonatal deaths arise in low income and middle income countries and about half occurred at home [3]. Globally the main direct causes of neonatal deaths are estimated to be pre-term birth (28%), severe infection (26%) and asphyxia (23%).

Neonatal tetanus accounts for a smaller proportion of deaths (7%), but is easily preventable. Low birth weight is also an important indirect cause of death [4].

WHO and UNICEF have developed the Integrated Management of Childhood Illness (IMCI) strategy to reduce childhood illness and deaths and improve growth and development of children [5]. Within the framework of that five-year strategic plan (2005-2009) for child health development, the IMCI strategy will be implemented in 4 model districts including Pyay District with more emphasis on improving family and community practices, especially about neonatal care. Before implementing the initiative in these districts, there is a need to assess the family and community practices

about neonatal care, and the barriers to these practices to provide baseline information for later evaluation and inputs for improved program design.

Objectives

The study aims at assessing the level of and barriers to key family and community practices concerning newborn care in Pyay District.

Specifically the study aims:

- to determine the level of the existing family and community practices concerning essential newborn care
- to identify barriers to the family and community practices concerning essential newborn care
- to provide baseline information and recommendations for improving the family and community practices of essential newborn care

MATERIALS AND METHODS

Study design

The study comprises 2 components:

1. Quantitative assessment and
2. Qualitative assessment.

Study area

The study was done in Pyay District including the following 5 townships:

1. Pyay Township
2. Pauk-khaung Township
3. The-gone Township
4. Shwe-taung Township
5. Paung-de Township

Study period

January to June 2005

Sample selection and sample size

In this study, for the proportion of mothers knowing danger signs of acute respiratory infection and diarrhoea is conservatively estimated at 50 percent (0.5). The z statistics is 1.96. Accuracy level is put at 0.03 level. The sample size is then calculated as:

$$n = (z^2 pq) / d^2$$

$$n = \{(1.96)^2 \times (0.50) \times (0.50)\} / (0.03^2)$$

$$= 1067$$

$$\approx 1100$$

A total of **1100 mothers with children under 5 years** were selected for the whole Pyay District.

Cluster sampling method was used for participant selection. Each cluster composes 11 mothers and 100 clusters were chosen to meet the sample size requirements.

11 Mothers (households) x10 Villages (clusters)x 2 RHCs x5 Townships = 1,100 Mothers

For qualitative data collection, the following methods and dimensions were used in each township.

a. Focus Group Discussions (FGDs),

2 sessions of FGDs (each session comprising 8-12 mothers of children between 1 year and 5 years)

2 sessions of FGDs (each session comprising 8-12 mothers of children under 1 year)

b. Key Informant Interviews (KIIs)

3 KIIs (with a midwife, an auxiliary midwife and a traditional birth attendant)

c. In-depth Interview (IDIs) with mothers of neonates

d. Observations were done on selected family practices relating to neonatal care.

Data collection

A pre-tested structured questionnaire was developed to assess the level of the family and community practices of newborn care. For qualitative data collection, pre-tested guides for IDIs and FGDs with mothers and for KIIs with health care providers were used for data collection.

Ethical consideration

After thorough explanation of the purpose of the study, informed consent was obtained from local authorities and all interviewees.

Data management and analysis

For quantitative assessment

Interviewers performed daily checks on the questionnaires for missing, inconsistent or illogical data and took action immediately. Field supervisors rechecked the forms and if any unclear or inconsistent findings observed they immediately called the interviewers for clarification and action. Then the field supervisor signed the questionnaires and sent it to the data manager. Data manager cleaned the data while entering data into the computer using Epi Info program and the data were analyzed using the Stata version 7 software.

For qualitative assessment

For FGD sessions, the note-takers jotted down the conversations as well as recorded them by tape recorders. For KIIs and IDIs the investigators themselves recorded the conversations manually. In the same evening of the day FGDs, KIIs and IDIs were conducted, the investigators and the note-takers completed their transcripts. Transcripts were checked against the tape recorder. The team leader coded the findings of interviews according to the main themes together with all the team members. While coding, any unclear phrase or sentence was clarified immediately. All team members sat together and matrix analysis was done manually. Comparisons were made between different groups of mothers, and recurring patterns and themes were noted down. Plausibility to draw any conclusion was observed. Then the team leader combined the information using the rapid group thematic analysis approach. The barriers to key family and community practices were particularly explored with more emphasis on information of programmatic implication than that of mere ethnographical interest.

RESULTS

Characteristics of the respondents

The quantitative assessment was performed on 1100 mothers with at least one child under five years but the analysis was done

only on 1090 mothers because 10 questionnaires had incomplete and inconsistent data. The age of the mothers in the quantitative study ranged from 18 to 52 years and the mean age was 31 years.

Table 1. Characteristics of the respondents in the qualitative assessment of the study

Data collection methods	Study population	Number of participants
FGD (10 sessions)	Mothers with child under one year	80
FGD (10 sessions)	Mothers with child of one to five years	77
KII	Midwives (MWs)	5
	Auxiliary Midwives (AMWs)	5
	Traditional Birth Attendant (TBA)	5
IDI	Mothers with newborn babies	7
Observation	Mothers with newborn baby or sick child	8

Age of mothers in the FGDs ranged from 17 to 46 years. Age of marriage was as young as 13 and as old as 35 years old. Total number of children ranged from 1 to 7 with a mean of 2.3.

Place of Delivery

Table 2. Place of delivery of the youngest child

Place	Frequency	Percent
Government health facilities	170	15.6
Private hospital/clinic	34	3.12
Home	881	80.83
Others	5	0.46
Total	1090	100

Most of the respondents (81%) had their youngest baby delivered at their home. The reasons for having a home delivery was due to strong family and social support, less financial cost and no need for transportation to hospitals and health centers. There were also few deliveries that took place at a hospital, especially those that were considered at risk. Only when MWs were not available TBAs were called for or, in villages where AMWs existed, their services were taken. Some of the mothers referred the delivery room as a “chay-yin-kan” (ခွေရင်းခန်း) (meaning back room).

The room was swept and cleaned, and very few mothers washed the room with water. Some mothers made sure that this room would be well protected from wind.

“The wind can cause “*mee-yat-thway-sann*” (မီးယပ်သွေးစမ်း)... it means having a fever with rigors it may even cause death.”
(a 46- year-old mother of 7 children, Pyay Township).

On observing the delivery rooms for the mothers who had delivered recently (less than 11 days), the delivery rooms were found to have one entrance and without windows. Lighting was found to be poor (Photo 1).



Photo 1. A delivery room inside the house
Accoucheur for birth

Table 3. Accoucheur of the youngest children of the respondents

Accoucheur	Frequency	Percent
Doctor	117	10.73
Nurse	82	7.52
Lady health visitor	42	3.85
Midwife	328	30.09
Health assistant	2	0.18
Auxiliary midwife	113	10.37
Traditional birth attendant	404	37.06
Others	2	0.18
Total	1090	100

Over sixty percent of the respondents had their youngest baby delivered by a trained attendant. Traditional birth attendants delivered about one third of the youngest babies of the respondents. The practice

reported was that even in the presence of a MW, a TBA was also called for providing supportive care like preparation of traditional medicine used during puerperal period, washing clothes, preparing food for mother, etc.

During delivery, relatives and neighbors stayed near the mother and gave support.

“...neighbors came into the delivery room ...sometimes the delivery room was full of themthat encouraged me and gave me the strength for delivery ...”

(a 38- year-old mother of 5 children, Pyay Township)

Hygienic practices at delivery

During the quantitative assessment, over twenty percent of the mothers had experienced of using the delivery kit. In the qualitative assessment in Pyay Township, all the interviewees with under one-year- old child said that they were provided with a clean delivery kit by the MW during the ANC period. The mothers expressed their pleasure for receiving these kits and knew well of the contents.

“Receiving the little bag was good. It is clean and convenient for my delivery. I really appreciated it”.

(a 23-year-old mother of two children, Pyay Township)

In other townships, although clean delivery kits had not been distributed to expectant mothers, some of the interviewees with under one year old child had experiences of being delivered using the delivery kits.

Both MWs and TBAs washed their hands with water and soap before handling the delivery. Both MWs and TBAs brought scissors (or blades) and threads for cutting and tying umbilical cord. Mothers had to provide boiled water for sterilizing the instruments.

The qualitative findings also pointed out that one mother in Pauk-khaung Township informed that the TBA used a sharp bamboo slab to cut the umbilical cord, and put

turmeric powder on the umbilical stump. Two mothers from Shwe-taung Township reported that the TBAs used razor blades to cut the umbilical cord; however, turmeric powder was also applied on the umbilical stump.

The quantitative assessment showed that different methods of sterilization were used for the instruments of umbilical cutting. About half (52%) of deliveries where home instruments were used, these instruments were not sterilized at all. Over ninety percent of the children whose umbilical stump was applied something on, 38% used various kinds of indigenous medicine including turmeric power. The majority of mothers interviewed said that their babies were not weighed at birth.

The majority of the mothers reported no experiences of incision and stitching of their birth canals except for few mothers. Mothers stayed most of the time in the delivery room by the fire place for seven days regarded as the puerperial period. Common practices known as “*oak-pu-tike*” (အုတ်ပူတိုက်) and “*chway-aung*” (ချွေးအောင်း) which facilitate healing of the uterus and latter promotes sweating was done by majority of mothers in the study.

Newborn care

Clearing of airway

Almost all the babies cried spontaneously after delivery. Only a few babies did not cry immediately after delivery. A traditional practice by TBAs in Pauk-khaung Township, when a child does not cry immediately after birth was to wash the baby's placenta called “*ah-chin-shaw*” (အချင်းလျှော်) meaning pressing and hitting the placenta. This practice is based on the belief that the life of the baby may be left behind in the placenta. Another local practice among several mothers in the study was applying a small amount of chewed pepper seed into the baby's mouth and keeping the baby on side to get rid of mucous from the baby's mouth.

Thermal control

In the quantitative study, over fifty percent of the respondents reported their youngest babies were bathed immediately after birth. Positive change in practice of bathing was observed in the qualitative study, some mothers with children of 1-5 years reported bathing their babies immediately after birth. They considered that by doing so, it would clean away all the dirt's from their babies' bodies. Only a few mothers with children under 1 year reported this practice.

“I used to bathe the babies soon after delivery ... but, after I attended a 7- day training given by the health staff I no longer practiced it.”

(a 61- year old TBA of 30 years experience, Paung-de Township).

Over ninety percent of the babies were wrapped with a cloth within half an hour after delivery, among which two thirds were wrapped immediately after birth. Babies were usually wrapped with a cloth all over the body including their heads.

Most mothers kept their babies close to them while a few kept their babies in a “*sakaw*” (စကော) which is a circular tray woven with bamboo (Photo 2). A few mothers expressed the following beliefs relating to this practice:

- putting in a “*sakaw*” (စကော) prevents dumbness and blindness;
- it is easy to carry and move the baby with the “*sakaw*” (စကော);
- “*sakaw*” (စကော) provides the warmth comparable to that of a mother's womb.

Infection control

Infection control for clean delivery and cord care during delivery has been described in the hygienic practices of delivery section of the findings. No mothers (except 2) reported giving special care to the eyes of their new born babies in the qualitative study. Shaving of babies' hairs was done at the end of “meetwee” traditionally defined puerperium, i.e. 7 days after birth, either by



Photo 2. A baby in a “sakaw” (paum)

a TBA or a relative. This was in accordance with local traditional practice to get rid of birth hair.

Care of special children

Only a very few reported experiences of delivering small size babies in the qualitative study.

“My child was only 4 pounds at birth and was delivered by breech. I thought she couldn’t survive longshe didn’t cry at birth ...The midwife gave resuscitation and told me to give the baby morning sunlight for 3 days to prevent yellow coloration of skin ...at the age of about one month she gained weight....”

(a 24-year-old mother with one child, The-gone Township).

Illness during newborn period

Only few of the respondents (15% of all the respondents) said that their youngest baby had been ill during one month after birth. The common complaints were fever and cough. About 70% of the ill newborns got treatment outside home. Mothers with infants stated that they dared not treat a very young child by themselves. Therefore they consulted a health care provider when the child was very young.

“The child is very young and delicate.....I am afraid something might happen. I go straight to *Sayama* (referring to a midwife)”.

(a 38-year-old mother of an infant, Pyay Township)

Breast feeding

Majority (65%) of the mothers started breast feeding their newborn within half an hour after birth. Quantitative assessment showed that ninety-two percent of the respondents breastfed their youngest babies and 82% of the respondents fed colostrum to their babies.

In the qualitative assessment, almost all interviewees reported that babies were fed with breast milk immediately after birth as advised by health staff. Compared to the mothers with older children, mothers with infants expressed more about the specific reasons for colostrums feeding, such as colostrums possessed nutritional values as well as disease preventing values.

Table 4. Exclusive breast feeding to the youngest child

Type of feeding	Frequency	Percent
Only breast milk	356	35.49
Breast milk+water	527	52.54
Breast milk+honey	39	3.89
Breast milk+porridge	19	1.89
Breast milk+commercial milk	28	2.79
Breast milk+other liquid	34	3.39
Total	1003	100

Only one third of the breastfed children received exclusive breast feeding. The commonest type of additional liquid fed to their babies was water (53%). The majority of the interviewees practiced feeding water to their new born babies from the age of days. They prepared boiled and cooled water for this purpose. The reason for giving water was to relieve the baby from thirst and to make the baby’s stomach cool.

Demand feeding was practiced by all mothers. The majority of mothers interviewed did not mention about proper positioning for breast feeding. They said they had not been taught by any health staff on this particular positioning practice. Even in this situation, the sitting position was generally practiced for younger babies (less than 3 months) to prevent the babies’ nostrils being blocked by breasts. Some

mothers said that they kept a pillow underneath the baby's head while breast-feeding to prevent baby's head from becoming flat. For older children, various positions were adopted.

DISCUSSION

Eighty percent of the women in the study gave birth to their baby at home. The mother and the baby were usually confined to the delivery room for the whole puerperial period of seven days. The findings highlight that the room was protected from wind with a belief that a mother must be kept away from wind. Observations had confirmed that the ventilation and lighting were found to be poor in the delivery rooms.

Over sixty percent of the mothers gave birth to their youngest baby with trained birth attendants. Among them over thirty percent of the mothers delivered with midwives. One third of the deliveries were done by TBAs in the townships under the study. Therefore the role of TBAs in rural areas is still important where MWs are not available. Community and social support during delivery was found to be very high. This finding showed that community support during delivery would be of help for the mother and the child in case of emergency.

The pre-distribution of clean delivery kit to mothers was found to be very much appreciated by the mothers in Pyay Township. The pre-distribution of the kit ensures the clean delivery kit for anyone who delivered in the absence of a trained attendance. Although most of the deliveries used clean sterilized instruments either from the clean delivery kits or brought by the birth attendance, there is still practice of using unsterilized bamboo slab for cutting the umbilical cord by TBAs. The practice of putting "*Hsa-nwin*" (ဆန္ဒနီ) into the umbilicus was also found in few mothers. Therefore this practice may hinder the infection control for newborn care. Puerperial period is locally defined as the period from the birth of the child up

to the seventh day. This period was considered very important both for the mother and the child. A lot of traditional beliefs and customs surround this period. Common practices known as "*oak-pu-tike*" (အုတ်ပူတိုက်) and "*chway-aung*" (ချွေးအောင်) which facilitate healing of the uterus and latter promotes sweating, respectively, need more exploration. Since these practices involve heating the bricks in a fire near them in a closed room may make the babies more vulnerable to acute respiratory tract infection of the newborn.

Although some of mothers of 1-5 years old children mentioned that some of their children were bathed immediately after birth, all the mothers with infants stated that their newborn babies were not bathed immediately after birth. This changing trend of the bathing practice has been a positive indicator for better thermal control of newborn babies. Nearly seventy percent of the babies in the study were also wrapped with clothes from head to toe immediately after delivery. In the townships under study, most of the mothers kept their newborn babies near them or in a *sakaw* which is a tray woven with bamboo. Even though the child was put in a *sakaw*, when observation was made on the practice, good thermal control was seen.

A practice which needs to be strengthened for all trained birth attendants was to weigh the child at birth, because the majority of the mothers in the qualitative study stated that their babies were not weighed at birth. Shaving of the babies' hair at the end of puerperium was also seen as a common practice in the study. Although the majority of the mothers stated that a disposable knife or a razor blade was used, promotion of sterilization for this practice should be ensured. All the children in the study were breastfed immediately after birth. The importance of colostrums was well known by the majority of mothers interviewed. But only one third of the breastfed children received exclusive breast feeding. Along with breastfeeding the majority of the

mothers in the study fed water to their new born babies starting from the age of days. This showed that feeding water to the new-born child was a normal process and they did not consider that this was an interruption to the exclusive breast feeding practice. Thus enhancing education on exclusive breast feeding is still needed.

Recommendation

- Standard cleaning procedure for preparing delivery room at home should be educated to the community.
- Clean delivery kit should be made available for all expectant mothers.
- Community support during delivery should be sustained.
- Weighing of newborn immediately after delivery should be encouraged to all the birth attendants.

- Education of mothers on proper exclusive breast feeding method should be strengthened.

REFERENCES

1. The Lancet. The world's forgotten children. *Lancet* 2000; 361:1.
2. Lack RE, Morris SS & Bryce J. Where and why are 10 million children dying every year? *Lancet* 2003; 361: 2226-34.
3. United Nations. United Nations Millennium Declaration (A/RES/55/2). New York: United Nations, 2000.
4. Bryce J, Arifeen S, Pariyo G, *et al.* Reducing child mortality: can public health deliver? *Lancet* 2003; 362: 159-64.
5. WHO/UNICEF. Integrated Management of Childhood Illnesses (IMCI), The Information Package 1999.

**Contributing factors to treatment interruption in patients
with tuberculosis in the selected townships with high default rates**

**Htin Zaw Soe, *Ye Hla, **Win Maung, **Thanda Lwin,
**Thin ThinYee & **Saw Thein*

**Department of Medical Research (Central Myanmar)*

***Department of Health*

A cross-sectional descriptive study was conducted among sixty-three TB defaults using pre-tested structured questionnaires and thirty-one health personnel by indepth interviews in Bago (East) and Ayeyawady Divisions from December, 2006 to January, 2007 with a main objective of finding out contributing factors to treatment interruption for two consecutive weeks or more. The study revealed that over two thirds of defaults had an education level of primary or below. Thirty-nine defaults (61.9%) interrupted treatment for two consecutive months or more. Majority presented their reasons of defaulting - a relief after taking drugs for two months and suffering from side effects of anti-TB drugs. From health personnel's aspect, contributing factors were found to be mostly concerned with low health knowledge and poverty among the defaults and their intolerance to side effects of drugs. An effective health education programme with social and monetary supports from local authority and voluntary donors in a sustained action could ensure a low default rate and a high treatment success rate.

INTRODUCTION

Tuberculosis (TB) is an emerging disease which affects 1.86 billion people in the world in 2000 alone and each year 8.74 million develop TB disease and about 2 million die [1]. In South-East Asia Region the disease burden is 5.7 million cases, 34% of the global burden and about 600,000 deaths annually [2]. In Myanmar registered cases of all types of TB for 2004 cohort were 90,008 and deaths were 4,447 with a case fatality rate of 5% [3]. By seeing it twelve TB cases die every day. Therefore the magnitude of the disease is considerably large in the region as well as in the country that needs to curb it with all-out efforts using various means and ways. In Myanmar case detection rate of new sputum smear positive was 83% and treatment success rate (TSR) 84% in 2004. Therefore Myanmar will have to exert its utmost efforts to reach

or go beyond the WHO target TSR of 85%. One of the several possible causes which hinder the progress of TSR may be defaulting from anti-TB treatment. There are some townships with high default rates in Myanmar [3]. Therefore defaulting has to be circumvented by removal of its root cause(s) after they have been discovered.

The present study was conducted with the objectives of: (i) to identify the proportion of cases with treatment interruption in management of TB patients in selected townships in 2005- 2006, (ii) to find out the contributing factors to treatment interruption among TB patients by both quantitative and qualitative methods, (iii) to analyze the proportions of and contributing factors to treatment interruption in the study areas and (iv) to propose the policy implications arising from the study in development of future plan for control of TB.

MATERIALS AND METHODS

The study design was a cross-sectional descriptive study undertaken in Bago (East) and Ayeyawady Divisions, Myanmar from December, 2006 to January, 2007 among TB defaults ie. patients stopping treatment before completion [2] and health personnel. In this study a default is operationally defined as a TB patient who has interrupted his treatment for two consecutive weeks or more.

Firstly Bago (East) and Ayeyawady Divisions were selected purposively and then four townships in the former (Bago, Thanatpin, Taungoo and Oketwin) and two townships in the latter (Pathein and Kangyidauk) were chosen on the basis of high default rate (DR) and research feasibility in fieldwork. Secondly lists of defaults were obtained from Divisional TB Offices. The feasible sample size ($n=63$) was estimated about 50% of TB patients taking treatment (defaults plus non-defaults) (ie. $N = (1.96)^2 (0.5) (0.5) / (0.09)^2 = 119$ TB patients). Thirdly all 63 defaults, who took treatment during the period from January to November, 2006, interrupting treatment for two consecutive weeks or more were traced up to their houses and then interviewed by each of four well-trained research assistants using pre-tested structured questionnaires. Fourthly principle investigator himself performed in-depth interview with thirty-one health personnel including doctors, using an interview guideline and a tape recorder. The data collected by quantitative method were analyzed by SPSS version 10. Data by qualitative method were transcribed, translated into English and then analyzed.

RESULTS

Data obtained by quantitative method

Number of default and default rates (for two consecutive months or more) for 2004 cohort and study population (defaults) in the study areas are described in Table 1.

The study included 63 defaults and by disease category, 23 (37%) were category I (smear positive), 29 (46%) category I (smear negative), 1 (1%) category II and 10 (16%) category III. Demographic and socio-economic characteristics of defaults are described in Table 2.

It was found that 18 (28.6%) were current smokers, 7(11.1%) ex-smokers, 12 (19%) current alcohol drinkers, one (1.6%) ex-drinker and 9 (14%) smokers as well as drinkers. Regarding the level of knowledge and attitude of TB diagnosis and treatment among defaults excluding those under 18 years, 51 (92.73%) were below 40% of the score. All respondents did not know the names of anti-TB drugs. Concerning diagnosis and treatment seeking behaviour, 51(81%) knew that anti-TB treatment is available at people's hospitals, 24 (38.1%) at RHCs/ sub-centres, 22 (34.9%) at TB clinics and 11 (17.5%) at private clinics. On time interval between first onset of symptoms noticed and first treatment taken by defaults, majority – 38 (60.32%) had interval of more than one month. Duration of treatment interruption among defaults revealed that most of them- 39 (61.9%) interrupted for more than two months. The main reason of defaulting from TB treatment among defaults (consumer's or patient's side) was suffering from side effects of drugs and relief after taking for two months and others are described in Table 3.

Table 1. No. of default and default rates for 2004 cohort^a and No. of study population (default) (%) in study areas

Study areas (Township)	Default		No. of study popu- lation (default) (%)
	No.	Rates (%)	
Bago ^b	26	7	18 (29)
Thanatpin ^b	13	17	8 (13)
Taungoo ^b	1	1	4 (6)
Oketwin ^b	8	10	7 (11)
Pathein ^c	33	8	23 (36)
Kangyidauk ^c	5	11	3 (5)

a = NTP, Myanmar, 2005, b = Bago (East) Division ,
c = Ayeyawady Division

Table 2. Demographic and socioeconomic characteristics of defaults

Characteristics	Number (%) (n=63)
Residence	
Urban	34 (54)
Rural	29 (46)
Age(year),mean(SD),median	36 (19.35), 33
Sex	
Male	32 (50.8)
Female	31 (49.2)
Marital status	
Single	20 (31.8)
Married	36 (57.1)
Divorced/separated/widowed	7 (11.1)
Education	
Illiterate	9 (14.3)
3Rs	9 (14.3)
Primary	26 (41.3)
Middle	9 (14.3)
High	8 (12.7)
Graduate	2 (3.1)
Occupation	
Agricultural/ animal husbandry	5 (7.9)
Home industry	1 (1.6)
Business man	1 (1.6)
Government staff	3 (4.8)
Shopkeeper	5 (7.9)
Casual labourer	27 (42.9)
Dependent	20 (31.7)
Monk	1 (1.6)
Monthly family income (Kyat), 42,000 (26,000), 36,000 mean (SD), median	
Family size , median	5
Housing type	
Brick	3 (4.8)
Wooden	24 (38.1)
Bamboo with thatch-roof	36 (57.1)
Water supply	
Piped	2 (3.2)
Well (shallow/ deep)	40 (63.5)
Pond	15 (23.8)
River	6 (9.5)
Latrine	
Sanitary	38 (60.3)
Unsanitary	25 (39.7)
Waste disposal	
Proper	30 (47.6)
Not proper	33 (52.4)

Data obtained by qualitative method

Thirty one health personnel were interviewed and the data obtained were not fully representative of other areas. Responses of some subjects are as follow.

“The reason of interruption in anti-TB treatment among TB patients is economic reason. Another reason is that defaults are mobile workers and they travel out without reporting to Basic Health Staff concerned.

Table 3. Reasons of defaulting from TB treatment among defaults (consumer's side)

No.	Reasons*	Number of default (%)
Economic		
1	Lack of traveling expense to go to clinic	13 (21)
2	Traveling for work	4 (6)
3	Economic difficulties	1 (2)
Social		
4	Nobody to accompany when going to clinic	2 (3)
5	Lack of money and ill child at home	1 (2)
6	Elopement	1 (2)
Attitude related		
7	Relief after taking drugs for two months	29 (46)
8	Perception that going to TB clinic is not necessary	12 (19)
9	Perception that they need not to take anti-TB drugs	10 (16)
10	Lack of anti-TB drugs at home	7 (11)
11	Social stigma	4 (6)
12	Failed to take drugs due to perception that he has not TB	1 (2)
13	Forgot to bring drugs to work site	1 (2)
14	No relief by anti-TB drugs but other drugs from drug shop	1 (2)
Health service related		
15	Clinic is too far	13 (21)
16	Drug not delivered by TB clinic two weeks ahead on request to sit for exam in other town	1 (2)
17	X-ray department closed	1 (2)
18	Hospitalized due to pneumothorax	1 (2)
Medicine related		
19	Suffering from side effects of drugs	37 (59)
20	Alcoholics afraid of interaction between alcohol and anti-TB drugs	2 (3)
21	Skin turning to dark colour	1 (2)
Other		
22	Co-infection with malaria	1 (2)
23	Presence of liver disease	1 (2)
24	Abortion	1 (2)

*One individual may have more than one reason

Some patients perceived that they become well after taking anti-TB drugs for some months and they do not come to health centre to obtain the drugs especially in the rainy season with heavy rainfall and floods in their living areas. The other is family problem like that patient went away beyond the living area for the purpose of his relative's illness or death.”

(A Divisional TB Officer)

“An increase in default rate in this township is due to weakness of DOTS. Anti-TB drugs are in the form of fixed-dose combination and it is not suitable for low-body- weight

person leading to overdose of some degree. Because of it patients suffer from effect of overdose like dizziness and weakness. Some patients lack in awareness of anti-TB treatment and peer education should be delivered among them.”

(A Head of Township Health Department)

“Defaults cannot bear the side effects of anti-TB drugs. Category I TB patients take drugs and are relieved and then they think that they have cured and take no more drugs and turn to work. Another thing is inaccessibility to health centres to take out drugs by the patients.”

(A Medical Officer of Station Hospital)

“TB defaults are not permanent residents. If they move to other places they do not report to the health centres and so are recorded as defaults here. Some are very poor and they cannot afford even traveling expense (eg. trishaw fees).”

(A lady health visitor of Urban Health Centre)

“The reason of becoming defaults is that they lack in health knowledge and cannot understand about anti-TB treatment. Some are afraid of being looked down and TB is regarded as a social stigma.”

(A midwife of Rural Health Centre)

By analyzing qualitative data the factors contributing to treatment interruption or defaulting could be categorized into three groups (Table 4).

DISCUSSION

Defaulting from anti-TB treatment is found to be one of the causes of low TSF in TB control programme. In this study all townships except Taungoo had DRs more than Union figure of 6% [3]. Therefore the default-tracing programme should be promoted in these townships. Thailand studies showed that DR was 30.5% in a respiratory clinic of a tertiary hospital, Bangkok [4], 17.9% at urban health centres,

Table 4. Factors contributing to treatment interruption (provider's side)

1. Patient related factors

Socioeconomic

- Low health knowledge
- Casual mobile labourers
- Poverty
- Devoting to and traveling for work/ business only
- Family problems
- Move to other places

Concept

- Reporting wrong addresses
- Failure to report to health staff
- Stubbornness
- Failure to continue drugs when a temporary relief felt
- Perception that they have no TB
- Social stigma

Other

- Presence of other co-existing diseases
- Religious reason

2. Health service related factors

Health centre/ clinic situated out of reach of patients

Staff (overworked/ uninterested)

DOTS system weakness eg. lack of provider

3. Treatment related factors

Prolonged duration of treatment

Side effect of drugs

Relative overdose effect of fixed-dose combination of anti-TB drugs

18.3% at a clinic specializing TB and 21.5% at a private hospital [5]. So DRs in the present study were lower than those in Thailand studies.

Among defaults, 46 (74%) were at the age between 20 to 59 years, a productive age group, so this might decrease in their work efficiency. Majority - 44(69.9%) were at the education level of primary or below including only three children who had not attended school yet. Therefore they were noted low-educated persons and should be educated repeatedly by a participatory approach till they become mature to adhere treatment. On occupation most of them were casual labourers and dependents and they have a low family income, making the family struggle for day to day survival. Smoking and drinking alcohol were the factors contributing to defaulting and the

odds of defaulting were 3.4 times higher among those who smoked and drank than those who did not [6]. Their knowledge and attitude score levels on TB were found to be low, favouring a chance of becoming a default. On seeking treatment, only about one third noticed that it is available at TB clinic and RHCs. Some 54 (85.7%) knew that the drugs are provided free of charge but only 20 (31.7%) had heard about the word 'DOTS'. Therefore they should be thoroughly explained about it. The interval between first onset of symptoms noticed and first treatment received showed that 38 (60.32%) had a delay of one month or more for anti-TB treatment. In this regard corresponding delay (median delay) in one of the studies among drug-resistant TB (DRTB) cases was 28 days [7]. Therefore defaults in the present study may probably have a chance of developing DRTB. Due to various reasons already mentioned above 39 defaults (61.9%) had interrupted their treatment for more than two consecutive months. So the default tracing programme in the study areas should be strengthened.

Regarding factors contributing to treatment interruption, from consumers' side, it is clearly shown that majority of the study group defaulted because of a relief after taking drugs for two months and suffering from drug side effects. The latter was also found as a factor in a study where the odds of becoming a default were 2.8 times higher in patients suffering from side effects than those who did not [6].

Factors discovered from providers' side socioeconomic problems, wrong belief and misconception among the defaults and some defect in health service system - all were culprits in occurrence of defaulting which may cause treatment failure. One of the studies depicted that patients who were absent from TB clinic were found to be 5.95 times more likely to have clinical treatment failure than treatment success [8]. The above-mentioned findings may help the health planners and policy makers in control of TB to a certain extent.

To combat the deadly disease and ensure high TSR, the following suggestions are made. These are: (i) Effective Health Education Programme including participatory approach, adequate counseling of patient and relatives and peer cohort review should be initiated and performed in a sustained action; (ii) Social and monetary supports should be contributed to ensure complete treatment by local authorities and voluntary donors; (iii) Proper transfer mechanism and opening of transit health centres should be created to enable to supply the drugs continuously especially to migrant TB patients; (iv) Drug should be supplied only after patient's address is confirmed by local authorities and health personnel concerned and alternative address is acceptable for mobile workers and (v) Cooperation between BHS and TB Office should be strengthened with a revitalization of existing default tracing programme.

ACKNOWLEDGEMENT

We would like to thank Director-General, Department of Medical Research (Central Myanmar) for his kind permission to conduct the study; Heads of Divisional Health Departments, Bago (East) and Ayeyawady Divisions for allowing the research works; and basic health staff and subject defaults who helped us enthusiastically.

REFERENCES

1. Kumaresan J. Epidemiology. In: *Tuberculosis, Epidemiology and Control*, Narain JP. eds., New Delhi, India: WHO. 2002; 15 – 33.
2. WHO. *The regional strategic plan for TB control 2006- 2015*. WHO Regional Office for South-east Asia. New Delhi, India. 2006; 63.
3. National TB control programme, Myanmar. *Annual report 2005*. Myanmar. 2006; 123.
4. Jittimanee SX, Kateruttanakul P, Modigan EA, et al. Medical characteristics and tuberculosis treatment outcomes in an urban tertiary hospital, Thailand. *The South-east Asian Journal of*

- Tropical Medicine and Public Health* 2006; 37 (2): 338 - 344.
5. Rim KI. Tuberculosis treatment default rate and its contributing factors: comparative evaluation between various health care settings in Bangkok. Bangkok. *Master Thesis*. Chulalongkorn University 2005; 146.
 6. Thuzar Han. Treatment outcomes, drug resistance and risk factors for smear positive pulmonary tuberculosis cases in Yangon Division, Myanmar in 2002. *PhD Thesis*. Science of Nursing and Midwifery, Faculty of Health Sciences, La Trobe University, Bundoora, Victoria 3086, Australia. 2005.
 7. Saw Thein. Drug resistant tuberculosis among patients treated in Union Tuberculosis Institute (Aung San) Yangon. *M.Med.Sc. Thesis*, University of Medicine I Yangon. 2002.
 8. Srisaenpang S, Pinitsoontorn S, Singhasivanon P, *et al*. Missed appointments at a tuberculosis clinic increased the risk of clinical treatment failure. *The South-east Asian Journal of Tropical Medicine and Public Health* 2006; 37(2):345-350.

Hepatitis B surface antigen sero-prevalence in a township in the north-eastern border region of Myanmar

Khin May Oo, *Ohmar Lwin, **Sandar Nyunt, *Aye Aye Yee,
San San Oo, **Khin Win, **Khin Pyone Kyi & *****Myo Khin*

**Experimental Medicine Research Division*

***Blood Programming Research Division*

****Nuclear Medicine Research Division*

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

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

Viral hepatitis B is a major public health problem in Myanmar with a carrier rate of 9-11% in the general population. Although many reports on the prevalence of hepatitis B exist, little is known about the epidemiology of viral hepatitis B in the border regions of Myanmar. A community-based study was carried out on 349 subjects (age 1–70 years) from Muse Township, near the Myanmar-China border. The DMR HBsAg ELISA kit was used to detect the hepatitis B surface antigen (HBsAg) in the sera samples of the subjects. The overall prevalence rate of hepatitis B seropositivity was 13.2%. There was no significant difference in hepatitis B sero-prevalence rates between males (13.9%) and females (12.7%). The HBsAg prevalence rate was significantly higher in 315 persons over the age of 10 years compared to 34 children below 10 years of age (14.6% vs 0%). Chinese subjects had the highest seropositive rate (24.1%) compared to Bamaras (14.3%), Shans (13.7%), Kachins (10%) and Palaungs (8%). A significantly higher HBsAg seroprevalence rate was observed in those with tattoos (23.9% vs 11.5%, $p=0.02$). Higher prevalence rate of HBsAg was observed in a border town as compared to the general population.

INTRODUCTION

Hepatitis B virus (HBV) infection is an important health problem worldwide, because the infection can cause significant liver diseases that range from fulminating hepatitis to hepatocellular carcinoma. Globally, more than two billion people have been infected with HBV and 350 million of them became chronic as detected by the presence of hepatitis B surface antigen [1].

It has also been reported that there is a wide intra-national variation in the prevalence of HBV infection in different areas of the same country. The prevalence of HBV infection is consistently higher in rural rather than urban areas. There have been reports on the ethnic and gender differences [2].

Hepatitis B virus infection is hyper-endemic in Myanmar and is a major health problem in the country. The magnitude of the problem of hepatitis B infection in Myanmar was reported in a large-scale field study carried out in both lower and upper Myanmar where 10.4% of the study population was found to be sero-positive for hepatitis B surface antigen (HBsAg) [3].

Subsequent studies carried out among different population groups revealed HBsAg carrier rate of 10-12% [4]. However, these studies were carried out on populations residing in major cities of the country. Based on the information that a certain area in Myanmar had a relatively higher HBsAg sero-positivity rate than the national figure [5], intra-national differences in HBsAg sero-positivity could exist in Myanmar.

Moreover, there is no information on HBsAg carrier rates of populations residing in the border regions of the country. It is assumed that the HBsAg carrier rates of the neighboring countries would be more or less reflected on the populations residing across the border. This study was carried out with the aim of studying the epidemiology of hepatitis B infection in different regions of Myanmar and was carried out in Muse Township in the eastern border region of Myanmar near the Myanmar-China border.

SUBJECTS AND METHODS

A community based cross-sectional study was carried out in September 2002, on 349 subjects residing in Muse Township who agreed to participate in the study. The study population included 137 males and 212 females within the age range of 1-70 years. The study population included Bamars, Shans, Kachins, Palaungs and Chinese subjects residing in Muse Township. After obtaining voluntary consent, a pre-tested questionnaire was used to collect social and biological data from each subject. Clinical and family histories were carefully asked and recorded. Two milliliters of venous blood were collected from each subject. The sera samples were centrifuged at 3000 rpm for 10 minutes and stored at -20°C and transported to the Department of Medical Research (LM) on ice packs for determination of hepatitis B surface antigen (HBsAg) using the in-house DMR hepatitis B surface antigen ELISA kit according to the Instruction Manual [6]. Data entry and analysis was carried out using MINI TAB 14 Statistical Package, USA. Univariate analyze were carried out to describe the data and Chi squared tests were carried out to determine the bivariate differences.

RESULTS

Age, sex and study groups

Females constituted 60% of the study population. The mean age of the subjects was

29.37 ± 13.74 yrs. There was no significant difference in age of the male and female subjects (29.37 ± 13.74 years vs 29.37 ± 13.74 years). Of the study population, 46 subjects (19 males, 27 females) were found to be HBsAg positive. Thus, the overall HBsAg prevalence rate was found to be 13.2%. There was no significant difference in HBsAg sero-positivity between males (13.9%) and females (12.7%). The HBsAg prevalence rate was significantly higher (0% versus 14.6%, Chi square value 5.05, $p = < 0.05$) in persons over the age of 10 years ($n= 315$, 14.6%) compared to children below 10 years of age ($n=34$, 0%). None of the children aged 1-10 years and those older than 50 years of age had HBsAg sero-positivity. The HBsAg sero-prevalence rate was significantly higher in adults of 31-40 years of age compared to that of children under 10 years (21.4% versus 0%, Chi square 14.22, $p= 0.014$). HBsAg prevalence rates in different age groups are shown in Figure 1.

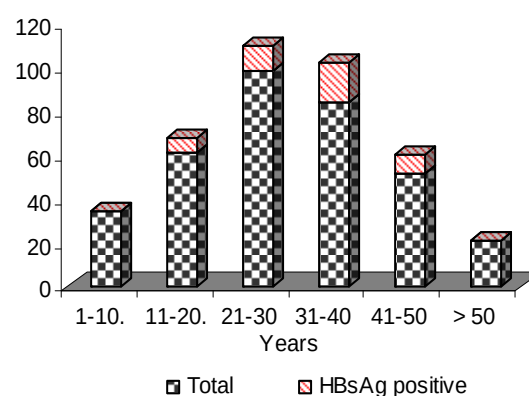


Fig. 1. Seropositivity of hepatitis B surface antigen by age groups

The HBsAg prevalence rate was 14.3% in Bamars, 13.7% among the Shan ethnic groups, 10% in Kachins, 7.9% in Palaungs and 7.1% in others. The highest prevalence rate of 24.1% was seen in Chinese subjects residing in Muse Township although they could not be classified as an ethnic group (Table 1).

Associated factors

The HBsAg sero-positivity rate was 23.9% in persons with tattoos and was significantly

Table 1. Hepatitis B surface antigen prevalence in study different groups

Study groups	Total	HBsAg (-)	HBsAg (+)	Percentage
Chinese	29	22	7	24.1
Bamar	77	66	11	14.3
Shan	131	113	18	13.7
Kachin	60	54	6	10.0
Palaung	38	35	3	7.9
Others	14	13	1	7.1
Total	349	303	46	13.2

higher compared to persons without tattoos (23.9% versus 11.6%, Chi Square value = 5.333, $p = 0.021$). In this study, there was no significant association of HBsAg sero-positivity with history of receiving blood transfusions (15.4% versus 12.9%), with surgical procedures (14.9% versus 12.5%) and liver disease (14.0% versus 13.0%). HBsAg sero-prevalence and associated factors are shown in Table 2.

Table 2. Associated factors of hepatitis B surface antigen (HBsAg) sero-prevalence among subjects residing in Muse Township

	Tattoo		Trans-fusion		Surgery		Liver disease	
	Yes	No	Yes	No	Yes	No	Yes	No
No. of subjects	46	303	39	310	101	248	50	299
HBsAg negative	35	268	33	270	86	217	43	260
HBsAg positive	11	35	6	40	15	31	7	39
Percentage	23.9	11.6	15.4	12.9	14.9	12.5	14	13

DISCUSSION

A wide variation of HBsAg prevalence rates has been reported by different authors, and the prevalence rates depended on different study populations and different geographical locations. The prevalence of chronic HBV infection is endemic in many countries of Asia and Africa and it has been estimated as 2.8% in developed countries and 7.6% in developing countries. HBV infection in the Asia-Pacific region is among the highest in

the world, and chronic HBV infection in most of the countries of the Asia-Pacific region is high (>10% prevalence) [7]. In the Asia-Pacific region, the carrier rate was less than 1% in Australia and New Zealand, 1-5% in Japan, Singapore, India and Thailand, 6-10% in Bangladesh, Indonesia, northern China and more than 10% in Taiwan, Southern China, Korea, the Philippines and Micronesia [2].

The findings from this study showed that the overall HBsAg prevalence was 13.2% in Muse Township situated on the Myanmar-China border in the north-eastern region of the country. Although the HBsAg prevalence rate in Muse Township was slightly higher than the HBsAg prevalence rates in previous studies it was much lower than the prevalence of HBsAg in residents of Namtee, a town in Kachin State which was 20.5% [5].

This intranational variation in the prevalence of HBV infection could be due to ethnic differences or local customs leading to iatrogenic transmission of HBV infection. Among a group of novices and monks of a monastery in a peri-urban area of Yangon, a high prevalence of HBsAg (17.5%) was detected and might be due to the common sharing of razor knives (*thin-done-dah*) used for shaving the head periodically [8].

One of the significant finding was the low HBsAg sero-positive rate in children under 12 years of age (2.4%). The HBsAg prevalence was lower than the previous reports on hepatitis B sero-positivity in children residing in the urban and peri-urban areas of Yangon. It had been reported that 8% of 189 children aged 3 months to 12 years from Mayangone Township were sero-positive to HBsAg [9].

In an earlier study by Khin Thant Zin in 1993, higher HBsAg sero-prevalence rates of up to 11% had been reported [10]. The difference might be due to temporal variation. Availability of hepatitis B vaccines could have reduced the prevalence

of HBsAg among the children. However, the proportion of children vaccinated with hepatitis B vaccine was not documented in the present study. It could be assumed that horizontal transmission predominate vertical transmission of HBV in the study population.

The findings from the present study showed that males had a slightly higher HBsAg carrier rate of 13.9% compared to 12.7% among the females. It was similar to the findings of a study by Khin Maung Tin where no significant difference in the prevalence for males (11.5%) and females (9.07%) was observed [3].

In a study by C-J Chen and co-workers, they had found that Taiwanese employees born in mainland China had rates of chronic HBV infection similar to those in the provinces of China from which they originated and was attributed to migrant difference [2]. Findings from this study had revealed that among the study groups, the Chinese subjects had the highest prevalence rate of 24.1% and reflect the HBsAg prevalence rate of 8- 20% in some parts of China [11].

Findings from this study had revealed that 23.9% of subjects with tattoos were positive for HBsAg which was significantly higher than the HBsAg sero-positivity rate in subjects without tattoos (23.9% vs 11.5%, $p= 0.021$). The common use of inadequately sterilized tattoo needles on different persons might be one of the iatrogenic causes of acquiring HBsAg sero-positivity.

There was no significant association of HBsAg sero-positivity with previous history of receiving blood transfusions (15.3% vs 12.9%) or surgical procedures (14.9% versus 12.5%). Screening of donor blood for hepatitis B surface antigen (HBsAg) at hospitals might have contributed to the prevention of accidental transmission of HBV to the recipients of blood transfusions. In this study, there was also no significant association of HBsAg sero-positivity with the presence of liver disease (14% versus

13%). Although the subjects gave history of liver disease, they did not have any information of being tested for hepatitis B markers and we were also unable to screen them for the presence of other HB markers at the survey.

In Muse Township, HBsAg sero-prevalence was higher in persons above of the age of 12 years which indicate a predominant horizontal transmission during childhood instead of the vertical transmission from infected mothers. Tattooing was the most significant factor associated with HBsAg sero-positivity. In order to prevent the transmission of HBV, hepatitis B vaccination programme of newborns and persons at risk should be augmented by health education activities for the control of hepatitis B infection in the north-eastern border region.

ACKNOWLEDGEMENT

We thank Dr. Soe Myat Swe, Township Medical Officer, Civil Hospital, Muse, Northern Shan State for his supervision and assistance.

REFERENCES

1. Global Health Situation and Projections and Estimates, 1992. World Health Organization, Geneva, 1992.
2. Chen CJ, Wang LY, Yu MW. Epidemiology of hepatitis B virus infection in the Asia-Pacific region. *Journal of Gastroenterology & Hepatology* 2000; 15(S): E3-E6.
3. 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.
4. Khin Pyone Kyi & Khin Maung Win. Viral hepatitis in Myanmar. *DMR Bulletin* 1995; 9(2): 1-31.
5. Win Maw Tun, Khin May Oo, Moh Moh Htun, San San Oo, & Sandar Nyunt. Prevalence of Hepatitis B surface antigen at Namtee, Kachin State. *Myanmar Health Research Congress Programme Abstract* Yangon, 2000; 7.
6. *Instruction Manual, DMR HBsAg ELISA Test Kit*. Department of Medical Research, Yangon, Myanmar.

7. Core working party for Asia-Pacific consensus statements on the prevention and management of hepatitis B and hepatitis C in the Asia-Pacific region. *Journal of Gastroenterology & Hepatology* 2000; 15: 825-841.
8. Khin May Oo, Khin Pyone Kyi, Moh Moh Htun, Sandar Nyunt *et. al.* Prevalence of hepatitis B markers in novices and monks residing in a same monastery. *Myanmar Health Research Congress Programme Abstract*, Yangon, 2001; 7.
9. Myo Khin, Aye Thiri Naing, Yi Yi Kyaw, Kyaw Soe, Ohnmar & Khin Ohmar Lwin. Sero-prevalence of Hepatitis B surface antigen and its associated factors in periurban children. *Myanmar Health Research Congress Programme Abstract*, Yangon 2001; 33.
10. Khin Thant Zin. A study of hepatitis B surface antigen among children of two areas in Yangon. Institute of Medicine I, *M. Med. Sc. Thesis* Yangon. 1993.
11. Zuckerman AJ, Hepatitis viruses. In: *Medical Microbiology*, Baron S, eds., 4th.ed. Galveston, TX, The University of Texas Medical Branch at Galveston 1996: 849-863.

Reproductive health needs of HIV positive women: client perspective

**Kyu Kyu Than, *Poe Poe Aung, **Htin Aung Saw, *Tin Tin Wai,
*The' Maung Maung & *Nyo Aung*

**Epidemiology Research Division
Department of Medical Research (Lower Myanmar)
**Department of Health*

This study aims to identify the reproductive health needs of HIV positive women from client perspective. A total of 20 women (12 married and 8 unmarried women) participated for serial in-depth interviews after obtaining written inform consent. The study was done from August 2006 to July 2007 in Waibargi Infectious Hospital, Yangon. Age of the respondents ranged from 19 to 43 years. The majority of women in the study had only one sexual partner in their entire life. Over two third of the women were diagnosed after their spouse. Almost all women in the study revealed their HIV status to their spouse immediately after diagnosis. Although all women accepted the positive status, women with children had better perception towards positive living. About half of the women in the study revealed that sexual relation still exist within their relation. Even though dual protection advantage of condom was recognized by women in the study, the gender power inequality to negotiate condom use with their partner was expressed by almost all women in the study. Apart from one woman who intends to have a child all others perceived that it was not fair and just to bring a life when one cannot even take care of one's health. Discrimination and stigma of being positive was more prominent among the unmarried women. This study highlights the need for comprehensive reproductive health care towards HIV positive women whether married or unmarried.

INTRODUCTION

HIV is the fourth largest cause of death globally and the challenge posed by HIV/AIDS is most critical in sub-Saharan Africa and Asia. In South-East Asia, Myanmar, along with Thailand and Cambodia, have been identified by UNAIDS as the three highest priority countries. In the early stages of the pandemic, HIV infection was predominantly among men. But estimates in December 2005 by UNAIDS indicate that about 40 million people are living with HIV of which about 17.5 millions are women.

According to the National AIDS Programme (NAP), UNAIDS and WHO, there were estimated 338911 adults living with HIV at the end of 2005. As sex dis-

aggregated national prevalence rates are currently not available, the sex ratio of 4 HIV infected males to 1 female has been estimated in 2004 by using government sentinel surveillance data of certain population groups. Since the decreasing trend of the sex ratio has been observed in many parts of the world, vulnerability of women being infected had risen in recent decade.

This increasing incidence of HIV/AIDS in women has clearly revealed their vulnerability which may be the consequences of social and economic inequality among women. Many studies have showed that more than 80 percent of women living with the virus were infected by their partners. The risk of mother to child transmission of HIV and subsequently leading to higher rate

of infant death also associated with maternal HIV infection and these issues highlight the need to know more about **“women with HIV infection”** there by reducing the risk not only for the HIV infected women herself but also to her future offspring.

Objective

- To provide information and understanding of the reproductive health needs of HIV positive women from client perspective for effective programming towards HIV positive persons.

MATERIALS AND METHODS

Study Design

A qualitative exploratory study

Study area

The study was conducted in Waibargi Infectious Hospital, Yangon from August 2006 to July 2007.

Data collection methods

Data collection activities were carried out between September 2006 and May 2007. Before starting the actual data collection, information regarding HIV positive women was gathered from Waibargi Infectious Hospital officials, persons working with PLWHA, people affected by HIV/AIDS and various local and government service providers. A broad outline for the In-depth Interview guide was developed.

Interviewers were thoroughly explained about the study's objective and sensitive nature of questioning towards sexuality issues with HIV positive persons. Only female interviewers were trained and used to gather information in this study.

A total of 20 serial In-depth Interviews (IDIs) were carried out with HIV positive women after obtaining a voluntary informed consent. Since the interviews were in-depth and sensitive, majority entail more than one session. Due to the reluctance of tape recording by some of the respondents, note

taking was done by the interviewers with permission. On average each IDI lasted about an hour and a half.

Ethical considerations

Ethical permission to conduct this study was sought and obtained from Institutional Ethical Review Committee, Department of Medical Research (Lower Myanmar).

Data analysis

All the IDIs sessions were jotted down by the interviewers. Transcripts were checked and discussed by the interviewers and the principle investigator on a daily basis. The principle investigator coded the findings of the interview according to the themes and sub themes together with the team members. While coding, any unclear phrases or sentences were clarified immediately. All the team members set together and matrix analysis was done manually. Comparisons were made between different people and recurring patterns of themes were noted down. Plausibility to draw any conclusion was observed. Then the team leader combined the information using the thematic analysis approach.

RESULTS

General characteristics of the respondents

A total of 20 women participated in the study. Using the marital status as main dimension, a total of 12 married and 8 unmarried (5 widows and 3 singles) women participated in the study. Age of the respondents ranged from 19 to 43 years. Majority of the women had above secondary school level of education. Six women were graduates.

Testing and perception towards HIV positive diagnosis

Majority of the women in the study had only one sexual partner in their entire life. Knowing the HIV positive result (HIV status) was an unforeseen event in their life. Along with astound and fear, most of the

women felt very negative about their husbands after knowing their status. Being naive and unknowledgeable about the disease and its transmission was regret as described by the women in the study.

“I was a dutiful housewife, I looked after my children, I was faithful to him and I took care of him well, my family was my life, I hate him, I will never forgive him.”

(A widow with a child)

Majority of the women in the study were out of the blue when they were told that they were positive. Some said it was like going to hell alive. The first few months were very painful as described by most of the women.

“I can recall those days and I was very grateful that I was able to overcome myself. It’s like a death sentence and getting to hell alive.”

(A married woman with 3 children)

With the belief of the religious teaching of ‘Kan’ (ကံ) and ‘Shay kutho’ (ရှေးကုသိုလ်) mostly expressed by many women meaning that this is their fate and they must have done something bad in their preceding life. Almost all women in the study were willing and accepting the condition as part of their life.

One educated woman described her incidence as “love blindness”. She was very well brad and educated and she had ample knowledge about the disease. But when she got married she was madly in love and she had totally elapsed.

“ Love is very powerful, at least for me at that time, but when I found out that I was positive during my pregnancy, I blamed my self first..... because...., why did I believe him.....? was he worth believing? ..., I don’t know.”

(A graduate woman)

The pregnant women in the study were diagnosed as HIV positive when they attended the pre-natal clinic at Central Women’s hospital. They had no prior discern about the disease. They did not have

any knowledge of the blood testing procedures. The results were given to them together with their family members. All were shocked at the time of diagnosis. According to the participants they did not get any type of counseling before the test from anyone.

“I went to the hospital with my husband and they gave us the paper and told me to go and see the doctor in the room. The doctor asks what my husband does and she said I have the HIV virus. ”

(A pregnant woman)

Disclosure of HIV positive status

For majority of the women, it took sometime to believe and accept their own HIV status. Over two third of the women in the study were diagnosed after their spouse. Almost all women in the study told their status to their husbands immediately after they were diagnosed.

“It’s because of him, of course he should be the first to know.”

(A married woman)

Disclosure of the HIV positive status to others apart from their spouse depends greatly upon their family structure, values and how they weigh the perceive consequences towards themselves and their family. Some of the HIV positive women said that they dare not to disclose their status to friends or close relatives out of fear and guilt and being blamed not only to her but also to her husband and children as well. Reluctance to tell older parents was revealed by most of the participants. Disclosure to the community especially to the closest neighbor was regarded as last by most of the respondents.

“They are already old and I don’t want them to live with shame and sadness because of me, so I told my sister not to tell them”

(A married woman)

“We keep it to ourselves, it’s our life and they will not and cannot do anything for us,

so why they tell which for sure they will disgrace us.”

(A married woman)

Revealing the HIV status was more difficult for a single woman than for a married woman. Women are expected to be demure and innocent before marriage. Value towards virginity is very high for a woman. Premarital sexual activity is regarded as a socially unacceptable behavior. Shame and disgrace for being sexually active puts a loosing face not only to the affected female but also to her family as well. Social stigma attached to HIV/ AIDS as a sexually promiscuous disease often enhances the single sexually active woman not to disclose her status.

“I don’t intend to tell anyone especially my parents and close friends. I’m really scared that they would find out, it will be a disgrace for my parents.”

(An unmarried woman)

Aspiration to live on as a positive person

Women in the study had more or less positive aspiration towards living with HIV. Determined and stronger aspiration to live was seen among positive women with children. Their responsibility as a mother and a care taker for their children was highly expressed by all the women.

“I need to be tough, strong and healthy so that my children will grow up well.”

(A woman with 3 children)

This intention to live on was also strongly seen among the mother who was recently diagnosed after her husband’s death.

“I have my children. He died because he was bad. As for me I still have to live for my children. I am not ashamed, I didn’t do anything wrong.”

(A 30- year-old widow with 2 children)

Sexual and reproductive life after diagnosis

About half of the women in the study revealed that they had sexual relations with their partners after diagnosis. But for

women they described that desire for sexual activities declined dramatically after diagnosis. For majority of the Myanmar women, sexual life starts along with marriage. Being married means submitted to a life time partner in her social, economical and environmental world. She gives herself to him with love, faith, commitment and is intended to stay with him in time of happiness as well as in times of difficulties. As she is submitted to her husband with love, the power to deny no to sex is not easy. This means that women in general have not enough power to resist to their partner’s need regarding sex. Majority said even though they had no feelings for sex and lack of intentions, the fulfillment of the partner’s need for sexual activity had to resume after about 6 months to one year. Thus desire towards sexuality was more or less partner driven.

“Both of us are positive, I have no desire since I know that I was positive. But because he wants it, and I am his wife, I had to give him.”

(A married woman with children)

Sexuality of unmarried HIV positive persons

Single unmarried females in the study were very regretful and had self blames and mixed feeling towards being sexually active without being married. Social norms that accept pre-marital sexual relationship in men and the social stigma being attached to being sexually active before marriage for women often leads towards non-disclosure for unmarried women. One of the females had only one sexual encounter in her whole life time. She was about to marry him because of the happening as she describes.

“He died of AIDS. So I went secretly to test myself and I found out that I was also positive (crying). Why me, my life is over.”

(An unmarried woman with a deceased partner)

“As I am a single, how would I show my face? People will assume I am a loose girl.”

(An unmarried woman)

Marriage and having children

The unmarried women in the study said marriage was out of the question for them. Apart from one woman who intended to have a child, all others perceived that it's not fair and justice to bring a life in when you cannot take care of yourself.

“Up to now I have no intention to get married. It's not fair to give 'misery' (ဒုက္ခ).”

(An unmarried woman)

“Even though we both are positive, I love children and there is still a chance of having a negative child. I want to take that chance.”

(A graduate woman)

Family planning including dual protection

Knowledge regarding family planning method among HIV positive women in the study was limited to condoms and only a few female mentioned about pills and injectables. Two married female with children also said that sterilization was a family planning method for HIV positive persons.

All the female knew that condoms were the most appropriate method for all the positive individuals regardless of their family planning intentions. They also mentioned that, as condoms are the only method that protects against HIV and other sexually transmitted infection, all providers recommend and encourage them to use condom. They also said that the providers rarely mentioned methods other than condoms to them.

“Sayama always told me to persuade my husband to use condom. She also provided me with condoms.”

(A married woman with one child)

Pattern of condom use

When inquiring about condom use and regularity, married women in the study said that they always try to insist and persuade their partners to use condom. But non-condom use during the sexual act was

experienced by almost all married women in the study. Together with 'thanaseik' (သနားစိတ်) meaning having a soft heart, love and commitment, the gender power dynamics within the sexual encounter often conquer the knowledge and awareness of the use of condom among HIV positive women in the study.

“When he started saying it is so pity that as a human, I cannot have children, his face and expression conquered my decision of using a condom.”

(A woman with 2nd marriage)

Prevention of Mother to Child Transmission (PMCT)

The pregnant women in this study had intended pregnancies. All were very happy with being pregnant before the diagnosis was made. They went to the hospital to take regular antenatal care service during their second trimester. They had no prior knowledge about the PMCT services and maternity care. All of the pregnant women in the study did not receive any pretest counseling prior to the HIV testing.

“I didn't know anything; they said blood test has to be done, so I took the blood test”

(A pregnant woman)

One of the pregnant women had received proper post-test counseling regarding prevention activities about her pregnancy and child birth.

“I now regret that I was pregnant, but no choice, it's my fate”.

(A pregnant woman)

Stigma and discrimination towards PLWHA

When the open question on “who do you think would be the most discriminated and stigmatized, a man or a woman?” Their opinions and answers were more or less similar. They regarded the disease as very shameful and disgrace. They said both men and women would be discriminated and stigmatized because of the positivity but the suffering compounded more to the woman in her social life. She faces tough time

because she not only bears the discrimination and stigma but also has to carry on the role as a care given for her infected partner and children. The discrimination and stigma of being HIV positive without being married had a significant impact on women.

“No matter who stigmatize or discriminate. Because we are women we are bound to take care of our husbands and children. We bear more burdens.”

(A married woman)

DISCUSSIONS

As HIV/AIDS in Myanmar is considered an advanced epidemic, people living with HIV virus at the end of 2005 was estimated to nearly forty thousand by the National AIDS Programme in 2006. As majority of the infected people are within the reproductive years, this study aims to shed light on the future care and support towards people living with the HIV/AIDS in consideration of their reproductive intention and needs from a female perspective.

All the married women living with HIV in this study revealed that they were infected from their partners. It was beyond their expectation and knowledge within marriage that this would happen. Their faithfulness compounded by little knowledge about the disease had made them to be HIV positive persons.

The gender inequalities are seen in which women generally are less able than most men to decide over their bodies and lives, which increases their vulnerability to HIV/AIDS. This finding indicates that lack of knowledge towards the disease and being faithful to their life time partner is of no protection for such women.

In this study, majority of the women had only one sexual partner in their entire life that being vulnerable to HIV can simply mean married to a potentially or already infected partner. Therefore education regarding pre-marital counseling and

voluntary counseling and confidential testing of both partners before marriage should be encouraged to the never married people before they become sexually active.

Disclosure or revealing the positive status about the infection was much harder for unmarried participants than married participants. The never married sexually active women bears a greater burden of living as a HIV positive person. Being sexually active for an unmarried male is usually regarded as a manly behavior. But for the unmarried female the social expectation towards virginity and sexual exposure before marriage put them as disgrace and shameful. This social norm and double standard given to men may enhance more women with HIV positive status to not disclose their status to anyone including their potential partners. Therefore people working with PLWHA should consider the negative impact faced by the HIV/AIDS infected never married women and find ways and means to overcome these barriers.

Even though dual protection advantage of condom was recognized by women in the study, the gender power inequality to negotiate condom use with the partner was expressed by almost all married women in the study. As condoms and abstinence were the most commonly discussed and encouraged methods regardless of the intention of reproduction for the HIV positive persons, the role of the gender power relation should also be taken into consideration by the providers. Therefore, programmes and providers working with HIV infected people must recognize the difficulty of condom use by women and help women to develop the necessary skills to negotiate condom use.

In this study the area on PMCT is covered insufficient since there were only three pregnant women. Although the PMCT programme has been widely carried out by both government and non-governmental (local as well as international) the findings from this study highlights that the PMCT programme should expand on covering a

wide range of women especially those women from inaccessible areas with the programmes.

Even though this study's primary focus was on reproductive intentions, issues regarding stigma and discrimination of PLWHA were also addressed. As of other studies, the discrimination and stigma were most prominent among the unmarried women. In order to give comprehensive care towards people living with HIV/AIDS whether married or unmarried programmes should take into great consideration of not only their perceived reproductive intentions but also towards discrimination and stigma issues which cannot be separated.

REFERENCES

1. *Health in Myanmar 2006*, Ministry of Health.
2. HIV/AIDS projection and demographic impact analysis in Myanmar, NAP/WHO, 2005.
3. HIV/AIDS, FSNHIV 0106, Country office Myanmar, WHO/DOH/NAP.
4. Joint programme for HIV/AIDS in Myanmar progress report 2003-2004 and fund for HIV/AIDS in Myanmar (FHAM). *Annual Progress Report* April 2004 to March 2005, UNAIDS – Myanmar.
5. Meeting the sexual and reproductive health needs of people living with HIV. Guttmacher Institute and the joint United Nation programme on HIV/AIDS (UNAIDS)(2006)
6. Rego A, Nadkarni V. HIV/AIDS in India: An annotated bibliography of selected studies (1990-2000). The gender and reproductive health research initiative. TISS and CREA New Delhi, 2002.
7. Sexual and reproductive health needs of women and adolescent girls living with HIV (Research Report on Qualitative Findings from Brazil, Ethiopia and the Ukraine, July 2006, Engender Health/ UNFPA.
8. Sexual and reproductive health of women living with HIV/AIDS: guidelines on care, treatment and support for women living with HIV/AIDS and their children in resource-constrained settings. WHO/UNFPA.

Evaluation of *in vitro* antimicrobial susceptibility of *Neisseria gonorrhoeae* strains isolated from symptomatic men and women in Yangon

*Wah Wah Aung & **Kyι Kyι Thinn

*Bacteriology Research Division

Department of Medical Research (Lower Myanmar)

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

The antimicrobial susceptibility pattern of *Neisseria gonorrhoeae* varies from one country to another and may also change with time. Periodic monitoring of the susceptibility of *N. gonorrhoeae* strains is important in order to adopt empirical treatment strategies. Antibiotic susceptibility of 40 *N. gonorrhoeae* strains isolated from urethral swab and endocervical swab specimens of symptomatic men and women was evaluated by disc diffusion method and determination of minimum inhibitory concentration (MIC) using E test method at Bacteriology Research Division, Department of Medical Research (Lower Myanmar) from August 2006 to July 2007. The susceptibility results of both methods showed satisfactory agreement for ciprofloxacin ($\kappa=0.646$), ceftriazone ($\kappa=0.806$) and penicillin ($\kappa=0.536$). The tested *N. gonorrhoeae* isolates were susceptible to azithromycin (87.5%), cefixime (80%), ceftriaxone (>70%) and ciprofloxacin ($\geq 55\%$) which are currently recommended drugs for gonococcal infections. High level ciprofloxacin resistance ($\text{MIC} \geq 4 \mu\text{g/ml}$) was found in 30% of tested isolates. The present study highlighted that majority of the tested *N. gonorrhoeae* isolates were susceptible to azithromycin, cefixime, ceftriaxone and the occurrence of high level ciprofloxacin resistant strains in the study population. Both disc diffusion and E test susceptibility testing methods gave reproducible results. Thus cost-effective and more feasible disc diffusion method could remain as the method of choice for routine diagnostic laboratories.

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 about 62 million new cases of gonorrhoea occurred worldwide in 1999 [1]. The onset of gonococcal infection is abrupt and the main symptoms are dysuria, frequency of micturition, urethral or cervical discharge. It infects only humans and causes lower genital tract infections, pelvic inflammatory diseases and related sequelae (infertility and ectopic pregnancy) in women, urethritis and epididymitis in men and proctitis, pharyngitis, conjunctivitis and disseminated infections in both sexes. It can

also be transmitted from an infected mother to a baby during child birth and causes ophthalmia neonatorum which can lead to perforation of the globe and blindness in the newborn [2].

The third generation cephalosporins, fluoroquinolones, spectinomycin and azithromycin are included for the treatment recommendation of gonorrhoea by the World Health Organization. The global health problem of gonorrhoea is concerned with the development of antimicrobial resistance in *N. gonorrhoeae* and Southeast Asia is the most likely origin of drug resistant *N. gonorrhoea*. The rate and pattern of antimicrobial resistance in *N. gonorrhoeae* vary with geographic regions and change over time. Therefore periodic monitoring of the

susceptibility of *N. gonorrhoeae* strains is important in order to adopt empirical treatment strategies [3].

A study carried out on 161 *N. gonorrhoeae* strains obtained from female sex workers in North Sumatra, Indonesia in 1996 showed that all strains exhibited plasmid mediated resistance to penicillin and/or tetracycline but all were susceptible to ceftriaxone, ciprofloxacin, kanamycin and spectinomycin [4]. Lawung *et al.*, found that multiple resistant determinants coexisted in a single isolate and double resistance to penicillin and quinolone were significantly increased among the isolates from patients attending Bangkok Hospital [5].

There are only limited studies on gonococcal antibiotic sensitivity pattern in Myanmar. Khin Mya Lwin *et al.* reported that 6.7% of gonorrhoea cases were detected among 71 pregnant women, 26 STD patients and 5 commercial sex workers and the gonococcal isolates were found to be less sensitive to penicillin, but susceptible to ciprofloxacin and ceftriaxone [6]. Gonococcal antimicrobial susceptibility of 14 isolates of *N. gonorrhoeae* isolated from married women in a community based study showed that 92.9% were resistant to penicillin, 85.7% were resistant to septrin, and 71.4% were resistant to amikacin and kanamycin [7].

Antimicrobial susceptibility testing of gonococcal isolates can be carried out by disc diffusion and determination of minimum inhibitory concentration (MIC) by agar dilution tests or E tests. Disc diffusion is a convenient method used by intermediate level laboratory in screening of individual isolates for antibiotic resistance. In disc diffusion test, discs containing known amount of an antimicrobial agents are placed on the surface of an agar plate seeded with tested *N. gonorrhoeae* strain. The antimicrobial agent diffuses into the medium, causing a zone of inhibition around the disc corresponding to the susceptibility of the strain to the agent [8].

Agar dilution technique is the reference method for determination of gonococcal MIC. The procedure is laborious and recommended for use only in central laboratories to provide epidemiological information. E tests provide an effective and simple alternative to reference agar dilution method for direct quantification of *N. gonorrhoeae* susceptibility. This method uses a plastic carrier strip containing a known gradient of an antimicrobial agent and calibrated to give results as MICs of the agent. E test strips are applied to the surface of a previously seeded agar plate and the MIC is recorded by reading the value at the point of intersection between the edge of the zone of growth inhibition and the strip [9].

Thus this study was carried out to determine the antimicrobial susceptibility pattern of currently recommended antibiotics for the treatment of gonococcal infection in Myanmar and to evaluate the antimicrobial susceptibility results of disc diffusion method compared with E test, so that a simple, reliable and cost-effective method could be recommended for antimicrobial susceptibility testing of gonorrhoea.

MATERIAL AND METHODS

Urethral swab specimens from 48 men presenting with urethral discharge and/or burning micturition and/or frequency of micturition, attending Central STD Clinic, Yangon and endocervical swab specimens from 124 women presenting with vaginal discharge and/or dysuria and/or pelvic inflammatory diseases, attending Central STD Clinic and out-patient clinics of Central Women Hospital and Thingangyan Sanpya Hospital were collected during August 2006 to July 2007. *Neisseria gonorrhoeae* strains were isolated by culture using modified Thayer- Martin media and chocolate agar. The isolates were confirmed by gram staining, oxidase test, catalase test, superoxol test, carbohydrate degradation test and Phadebact GC monoclonal antibody coagglutination test (Boule, Sweden).

Antibiotic susceptibility tests were carried out on 40 culture confirmed *N. gonorrhoeae* isolates using Gonococcal (GC) agar base with 1% IsoVitaleX supplement by disc diffusion method as recommended by Clinical Laboratory Standard Institute (CLSI) [8]. Antibiotic discs (Oxoid, Hampshire, England) containing penicillin G (10 IU), tetracycline (30 µg), chloramphenicol (30 µg), sulphamethoxazole/ trimethoprim (25 µg), gentamycin (10 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), cefixime (5 µg) and kanamycin (30 µg) were tested. The isolates were defined as susceptible, intermediate and resistant according to CLSI.

The susceptibility of penicillin, ciprofloxacin, ceftriaxone and azithromycin were detected by determination of MIC using E test method on 40 culture confirmed *N. gonorrhoeae* isolates. A fresh overnight culture of *N. gonorrhoeae* was suspended in Mueller Hinton broth until a turbidity equivalent to 0.5 McFarland standard was obtained (corresponding to 10^4 colony forming units per ml). The cell suspension was inoculated onto GC agar plate by gently spreading with a sterile cotton-tipped swab.

After drying of the plates for 3-4 minutes, the E test strips were placed in the centre of plate, and then incubated at 35°C in 3-5% CO₂ for 24 hours. The MIC values were determined by recording the point of intersection of the growth elliptic margin zone with the MIC scale value on the E test strip. Based on MIC values described by the manufacturer (AB Biodisk, Sweden), the isolates were interpreted as susceptible, intermediate and resistant [9]. Both disc diffusion and E test were performed simultaneously with the same inoculums. Reference *N.gonorrhoeae* ATCC 49226 was used as quality control for disc diffusion and E test. Kappa statistics was applied to determine the agreement between the two methods.

This study was approved by Research and Ethical Committee, University of Medicine (1).

RESULTS

N. gonorrhoeae strains were isolated from 12/48 (25%) men and 28/124 (22.6%) women comprising 40/172 (23.3%) of total study population.

Antibiotic susceptibility by disc diffusion method

The antibiotic susceptibility pattern revealed by using disc diffusion method is shown in Table 1. Although most of the isolates were sensitive to cefixime (80%), ceftriazone (75%) and ciprofloxacin (60.0%), they were resistant to older antibiotics like penicillin (80.0%), tetracycline (82.5%), chloramphenicol (82.5%) and septrin (77.5%).

Table 1. Antibiotic susceptibility pattern of *Neisseria gonorrhoeae* strains tested by disc diffusion method (n=40)

Tested antibiotics	Susceptible no. (%)	Intermediate no. (%)	Resistant no. (%)
Penicillin	4 (10)	4 (10)	32 (80)
Tetracycline	3 (7.5)	4 (10)	33 (82.5)
Gentamycin	8 (20)	12 (30)	20 (50)
Trimethoprim/ Sulphamethoxazole	4 (10)	5 (12.5)	31 (77.5)
Chloramphenicol	3 (7.5)	4 (10)	33 (82.5)
Ciprofloxacin	24 (60)	4 (10)	12 (30)
Ceftriaxone	30 (75)	-	10 (25)
Cefixime	32 (80)	2 (5)	6 (15)
Amikacin	20 (50)	4 (10)	16 (40)
Kanamycin	16 (40)	4 (10)	20 (50)

Zone inhibition diameter (mm) for interpretative criteria recommended by CLSI: penicillin (S ≥ 47, I= 27-46, R≤26), tetracycline (S≥38, I=31-37, R ≤ 30), gentamycin (S≥15, I=13-14, R≤12), trimethoprim/ sulphamethoxazole(S≥16, I=11-15, R≤16), chloramphenicol (S≥18, I=13-16, R≤12), ciprofloxacin (S≥41, I=28-40-46, R≤27), ceftriazone (S≥35), cefixime (S≥31), amikacin (S≥17, I=15-16, R≤14), kanamycin (S≥18, I=14-17, R≤13)

R= resistant, I= intermediate, S= susceptible

Antibiotic susceptibility determined by E test

The antibiotic susceptibility of penicillin, ciprofloxacin, ceftriaxone and azithromycin tested by determination of MIC using E test method is shown in Table 2. The suscep-

tibility revealed as: azithromycin (87.5%), ceftriaxone (72.5%) and ciprofloxacin (55.0%). Penicillin showed high resistant rate of 80.0%.

Table 2. Antibiotic susceptibility pattern of *N. gonorrhoeae* determined by E test

Tested anti-biotics (n=40)	Susceptible no.(%)	Intermediate no. (%)	Resistant no. (%)
Azithromycin	35 (87.5)	-	5 (12.5)
Ciprofloxacin	22 (55.0)	8 (20.0)	10 (25)
Ceftriaxone	29 (72.5)	-	11 (27.5)
Penicillin	3 (7.5)	5 (12.5)	32 (80.0)

AB Biodisk's Interpretative criteria according to MIC ($\mu\text{g/ml}$): azithromycin ($R \geq 2, I=1, S \leq 0.5$), ciprofloxacin ($R \geq 1, I=0.12-0.5, S \leq 0.06$), ceftriaxone ($S \leq 0.25$), penicillin ($R \geq 2, I=0.12-1, S \leq 0.06$)

R= resistant, I= intermediate, S= susceptible

Comparison of antibiotic susceptibility by disc diffusion and E test method

Antibiotic susceptibility to ciprofloxacin, ceftriaxone and penicillin by disc diffusion and E test methods are compared and shown in Table 3. Statistically moderate level of agreement for ciprofloxacin ($\kappa=0.646$) and penicillin ($\kappa=0.536$) and high level of agreement for ceftriaxone ($\kappa=0.806$) were found.

Table 3. Comparison of antibiotic susceptibility to ciprofloxacin, ceftriaxone and penicillin by disc diffusion and E test method

Antibiotics tested	E test suscep-tibility	Disc Diffusion Test Susceptibility			Total
		Suscep-tible no.	Inter-mediate no.	Resistant no.	
Ciproflo-xacin	S	20	1	1	22
	I	3	2	1	6
	R	1	1	10	12
	Total	24	4	14	40
Ceftri-axone	S	28	-	1	29
	I	-	-	-	-
	R	2	-	9	11
	Total	30	-	10	40
Penicillin	S	1	1	1	3
	I	2	2	1	5
	R	1	1	30	32
	Total	4	4	32	40

R= resistant, I= intermediate, S= susceptible

MIC values determined by E test

Anti gonococcal activities of azithromycin, ciprofloxacin, ceftriaxone and penicillin

tested by E test expressed in range of minimum inhibitory concentrations is shown in Table 4. High level ciprofloxacin resistance ($\text{MIC} \geq 4 \mu\text{g/ml}$) was seen in 10/40 (25%) of tested isolates.

Table 4. Range of MIC values ($\mu\text{g/ml}$) of penicillin, ciprofloxacin, ceftriazone and azithromycin by E test

Antibiotic (MIC range on E test strip)	Sensitivity pattern	No. of strains	MIC values of tested antibiotic
Azithromycin (0.016-256)	Susceptible	35	0.25-0.5
	Resistant	5	2-6
Ciprofloxacin (0.002-32)	Susceptible	22	0.032- 0.064
	Intermediate	6	0.125- 0.5
	Resistant	12	4-8
Ceftriaxone (0.002-32)	Susceptible	29	0.094 – 0.25
	Resistant	11	0.75-1.5
Penicillin (0.002-32)	Susceptible	3	0.064
	Intermediate	5	0.12-0.1
	Resistant	32	2-32

DISCUSSION

In the present study 40 *N. gonorrhoeae* strains were isolated from a total population of 172 symptomatic patients. Of these strains, 24/82 (29.3%) strains were isolated from STD clinic attendees (48 men and 34 women) and 16/90 (17.8%) were isolated from women attending gynaecological OPDs. The gonorrhoea patients were within the age of 24-37 years which is the reproductive age group. The majority (75%) were dependents, labourer and commercial sex workers. Only 23.5% of study population had the high school and university education. The similar finding was also seen in a study by Jolly *et al.* (1995) which stated core transmitters of gonorrhoea were young age, low socioeconomic status and high risk group for STDs eg. commercial sex workers [10].

The most common presentations were dysuria and urethral/vaginal discharge. Inguinal lymphadenopathy was seen in only 5% of patients and the systemic manifestation like fever, arthralgia and rash were seen in only one patient. Only 5% gave

history of previous STDs and 6% of patients had history of prior antibiotic treatment.

In the present study, there was satisfactory level of agreement for ciprofloxacin, ceftriaxone and penicillin between disc diffusion and E tests. This finding was compactable with the result reported by Bala *et al.*, [11]. Although E test MIC testing has advantage of producing more defined estimation of the level of resistance, it is expensive (approximately USD 15 for one test), disc diffusion which is simple, less expensive (approximately USD 8 for 50 tests) and feasible could remain the method of choice for routine testing.

The World Health Organization (WHO) recommended that antibiotic susceptibility testing should be performed against agents that are being used as primary therapies against uncomplicated gonorrhoea and agents that are used as alternative therapies. 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, ceftriazone, cefixime, azithromycin and spectinomycin. Kanamycin and trimethoprim/sulphamethoxazole are the drugs for the alternative regimen.

A large portion of gonococcal isolates worldwide are now resistant to penicillin, tetracycline and other older antimicrobial agents [3]. The present study also exhibited the high percentage resistance to penicillin, tetracycline, chloramphenicol, trimethoprim/ cotrimoxazole, gentamycin, kanamycin and amikacin. The majority of *N. gonorrhoeae* isolates were susceptible to azithromycin (87.5%), cefixime (80%), ceftriaxone (>70%) and ciprofloxacin ($\geq 55\%$). Recently, there were reports on the occurrence of high level resistance to ciprofloxacin ($\text{MIC} \geq 4 \mu\text{g/ml}$) in Argentina, Israel and Taiwan [12, 13, 14] and our study also found out 30 % of high level

ciprofloxacin resistant cases among the tested isolates.

In Khin Mya Lwin's study during 1998, 6.7% of the gonococcal isolates from STD patients in Hlaingtharya township in Yangon were found to be less sensitive to penicillin, but susceptible to ciprofloxacin and ceftriazone [6]. Thus decreased susceptibility of gonococcal isolates to ceftriaxone and ciprofloxacin were noted in the present study. Fluoroquinolone-resistant gonococci have appeared in several Asian countries since early 1990 [15]. Although quinolones such as ciprofloxacin are recommended as the first line of therapy for gonorrhoea, the emergence of significant resistance to ciprofloxacin and presence of high level resistant strains will limit the usefulness of this drug. According to the *in vitro* susceptibility results, azithromycin, cefixime and ceftriazone were found to be effective against the majority of tested *N. gonorrhoeae* strains. However, we could not report the susceptibility of spectinomycin as its susceptibility result on the reference quality control strain (*N. gonorrhoeae* ATCC 49226) showed inconclusive results.

Our study revealed the susceptibility pattern of currently recommended antibiotics used for the treatment of gonococcal infection and these results could provide useful information to National AIDS/STD Control Program in Myanmar. The present study also highlighted that majority of the gonococcal isolates were susceptible to azithromycin, cefixime, ceftriaxone and the occurrence of high level ciprofloxacin resistant strains in the study population. Both disc diffusion and E test susceptibility testing methods gave reproducible results, disc diffusion method could remain as the first choice as it is cost-effective and more feasible in routine diagnostic laboratories in developing countries.

ACKNOWLEDGEMENT

We would like to thank Dr. Kyu Kyu Than, Research Scientist, Epidemiological

Research Division, Department of Medical Research (LM) for statistical analysis. We are grateful to doctors, nurses, laboratory technicians and staff of Central STD clinic, Obstetrics and Gynecological Departments of Central Women's Hospital and Thingangyun Sanpya Hospital for their help in patient's recruitment and specimen collection and staff of Bacteriology Research Division, Department of Medical Research (LM) for laboratory testing.

REFERENCES

1. World Health Organization (2001). Global prevalence and incidence of selected curable STDs overviews and estimates. http://www.who.int/docstore/hiv/GRSTI/who_hiv_aids_2col_02pdf . Accessed on 12-4-06.
2. Brooks GF, Butel JS & Morse SA. The Neisseriae. In: *Jawetz, Melnick and Adelberg's Medical Microbiology* 23rd Edition . Lange Medical Brook / Mc Graw - Hill Medical Publishing Division, 2004; 295-304.
3. World Health organization Guidelines (2001). Guidelines for the management of STIs. http://www.who.int/mipfiles/2226/who_hiv_aids_2001.01.pdf. Accessed on 12-4-06.
4. Su X, Hutapea N, Tapsall JW & Lind I. Plasmid-mediated resistance of *Neisseria gonorrhoeae* strains isolated from female sex workers in North Sumatra, Indonesia, 1996. *Sexually Transmitted Disease* 2003; 30(2): 178-182.
5. 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* 2005; 4: 130-140.
6. 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 Tharyar Township. *Myanmar Medical Journal* 1998; 42 (3, 4): 38-42.
7. Mar Mar Nyein, Ba Thike K, Khin Thet Wai, Khin May Oo, Wah Wah Aung, *et al.* Non-ulcerative STDs among married women in selected urban and semi urban area. *Myanmar Health Sciences Research Journal* 2003; 15(1-3): 5-9.
8. Van Dyck E, Meheus AZ & Piots P. Gonorrhoea. In: *Laboratory diagnosis of sexually transmitted diseases*, WHO publication, 1999; 1-21.
9. Biedenbach DA & Jones RN. Comparative assessment of E test for testing susceptibilities of *Neisseria gonorrhoeae* to penicillin, tetracycline, ceftriaxone, cefotaxime, and ciprofloxacin: investigation using 510(k) Review Criteria, recommended by the Food and Drug Administration. *Journal of Clinical Microbiology* 1996; 34(12): 3214-3217.
10. Jolly AM, Orr P, Harmond G & Young TK. Risk factors for infection in women undergoing testing for *C. trachomatis* and *N. gonorrhoeae* in Manisota, Canada. *Journal of the American Venereal Disease Association* 1995; 22(5): 289-295.
11. Bala M, Ray K & Gupta SM. Comparison of disc diffusion results with mean inhibitory concentration (MIC) values for antimicrobial susceptibility testing of *Neisseria gonorrhoeae*. *Indian Journal of Medical Research*; July 2005 122:48-51.
12. Fiorito S, Galarza P, Pagano I & Oviedo C. Emergence of high level ciprofloxacin resistant *Neisseria gonorrhoeae* strains in Buenos Aires, Argentina. *Sexually Transmitted Infections* 2001; 77: 77.
13. Dan M, Poch F & Sheinberg B. High prevalence of high level ciprofloxacin resistance in *Neisseria gonorrhoeae* in Tel Aviv, Israel: Correlation with response to therapy. *Antimicrobial agents and chemotherapy* 2002; 46: 1671-1673.
14. Hsueh PR, Tseng S-P, Teng LJ & Ho SW. High prevalence of ciprofloxacin resistant *Neisseria gonorrhoeae* in northern Taiwan. *Clinical Infectious Diseases* 2005; 40: 188-192.
15. Ieven M, Van Looveren M, Sudidgoadi S, Rosana, Y, Goossens W, Lammens C, Neheus A & Goosens H. Antimicrobial susceptibilities of *Neisseria gonorrhoeae* strains isolated in Java, Indonesia, *Sexually Transmitted Diseases* 2003; 30(1): 25-29.

MYANMAR
HEALTH
SCIENCES
RESEARCH JOURNAL

DEPARTMENT OF MEDICAL RESEARCH (Lower Myanmar)
Ministry of Health, Yangon, Myanmar

MYANMAR HEALTH SCIENCES RESEARCH JOURNAL
INDEX TO VOLUME 19 (1-3), 2007

AUTHOR INDEX

A		Khin Pyone Kyi	32, 94, 121, 125, 161
Abe, Kenji	125	Khin Sandar Oo	69
Aung Than	103	Khin Saw Aye	6, 106
Aung Thu	76	Khin Thida Wai	76
Aye Aye Maw	24, 53	Khine Khine Lwin	97
Aye Aye Myint	37, 53, 87, 136	Khine Khine Phyu	141
Aye Aye Yee	53, 161	Khine Su Win	19
Aye Maung Han	115	Khine Win	161
Aye Myint Swe	111	Ko Ko Zaw	19, 147
H		Kyaw Kyaw	6
Han Win	76, 106	Kyaw Myint	19, 27, 47
Htar Htar Aung	24	Kyaw Nyunt Sein	27, 47
Htay Htay Hlaing	57	Kyaw Oo	27, 47, 69, 115
Htin Aung Saw	166	Kyaw Soe	141
Htin Zaw Soe	13, 155	Kyi Kyi Mar	43
Hyunah Kim	32	Kyi Kyi Thinn	173
J		Kyi Kyi Win Zaw	76
Jae Seung Kim	94, 121	Kyin Hla Aye	131
K		Kyu Kyu San	76
Khin Chit	97	Kyu Kyu Than	69, 147, 166
Khin Lin	103	L	
Khin Mar Aye	147	Le' Le' Win	19, 27, 43, 47
Khin Maung Lay	19	M	
Khin Maung Lwin	24	Mar Mar Nyein	24, 81
Khin May Oo	53, 81, 125, 161	Marlar Than	63
Khin Myat Tun	76, 106	Matsuoka, Masanori	6
Khin Nwe Oo	81	Maung Maung Mya	106
Khin Nyo	63	May Aye Than	97
Khin Ohnmar Lwin	125	Min Wun	97
Khin Phyu Phyu	131	Minn Minn Myint Thu	141
Khin Phyu Pyar	63	Moe Moe Win	24

Moe Thida	27
Moh Moh Hlaing	1
Moh Moh Htun	111, 125
Mon Mon	69, 147
Muya Than	53
Mya Aye	131
Mya Moe	103
Mya Thandar Win	141
Myat Mon Oo	141
Myat Phone Kyaw	63, 115
Myint Lwin	147
Myo Khin	53, 115, 161
Myo Myo Mon	69, 147

N

Nam Joong Le	94
Ne Win	111, 115, 141
Ngeow Yun Fong	81
Ni Ni Aye	76
Ni Ni	131
Nilar Tin	43
Nu Nu Win	97
Nyo Aung	166
Nyunt Thein	115

O

Ohmar Lwin	161
Ohnmar May Tin Hlaing	97

P

Paing Soe	115
Pe Than Htun	106
Peter Ne Win	19
Phyo Maung Thaw	57
Phyu Phyu Aung	1
Poe Poe Aung	166

S

S. Kyaw Hla	115
San Aye	113

San San Aye	43, 69
San San Oo	125, 161
San Shwe	19, 27, 47
Sandar Aung	53
Sandar Kyi	76
Sandar Nyunt	161
Saw Lwin	13, 103
Saw Thein	155
Saw Win	115
Sein Min	106
Soe Aung	69
Suk Hoon Ha	32, 121
Swe Win	136

T

Than Htein Win	115
Than Htut	63
Than Than Aye	97
Than Than Lwin	76
Than Tun Sein	43, 57, 147
Thandar Lwin	155
Thandar Shwe	1
Thaw Zin	63
The' Maung Maung	166
Thein Aung	147
Thein Kyaw	97
Thein Tun	103
Theingi Thwin	1
Theingi Win Myat	1
Thin Thin Yee	155
Thuzar Myint	24
Tiang Yee Peng	81
Tin Khine Myint	1
Tin Lin Myint	87
Tin Maung Tsoh	19
Tin May Nyunt	97
Tin Tin Aung	115
Tin Tin Wai	166

Tinzar Aung	43
Tun Aung Kyi	19
Tun Pe	37, 87, 136
W	
W. Tun Lin	13
Wah Wah Aung	24, 81, 173
Win Aung	32, 94, 113, 121
Win Maung	155
Win Myint	131

Win Win Myint	63
Y	
Yan Naung Maung Maung	106
Ye Hla	155
Ye Htut	53, 131
Ye Win Than	19, 27, 47
Yeon Hee Kim	32, 94, 121
Yi Yi Kyaw	53
Yin Thet Nu Oo	6

SUBJECT INDEX

A

ABO blood-group system	113
Aedes	13
Alpha-Thalassemia	141
Antibodies	53
Antihypertensive agents	97
Antimalarials	63, 131
Antivenoms	37
Awareness	69

B

Bacteriological techniques	24
Burns	24

C

Child growth and development	1
<i>Chlamydia trachomatis</i>	81
Constitution and by laws	131
Copper sulfate	13
Costs and cost analysis	43

D

Delivery	43
Disabled persons	19, 47
DNA, viral	125

E

Environmental exposure	1
------------------------	---

F

Family practice	147
Fetal blood	141

G

Genome, viral	125
Genotype	6
Glomerulonephritis	111
Glucosephosphate dehydrogenase	103

H

Hepatitis B surface antigens	32, 94, 121, 161
Hepatitis B vaccines	125
HIV seropositivity	166
Hypertension	97

I

Immunoenzyme techniques	111
Immunoglobulins	111
Infant, newborn	147
Inservice training	57

L

Labor	43
Larva	13
Lead Poisoning	1
Leprosy	6,19, 27, 47

M

Malaria	53, 103, 106
Malaria, falciparum	63
Medical waste disposal	115
Mefloquine	63
Midwifery	57
Mortality	87
<i>Mycobacterium leprae</i>	6

N

Needles	115
<i>Neisseria gonorrhoeae</i>	81, 173
Neoplasms	69

P

Plantago	97
Plants, medicinal	97, 131

<i>Plasmodium falciparum</i>	53	Snakebites	87, 113,
Poisoning	87		136
Polymerase Chain Reaction	81, 125	Social work	27
Polymorphism (Genetics)	6, 32, 94, 121	Swertia	131
		Syringes	115
R		T	
Recombination, genetic	94	Tuberculosis	155
Reproductive medicine	166	V	
Residence characteristics	27	Viper venoms	37
Rural health services	57	W	
Russell's viper	37, 113	Women's health	69
S		Y	
Self care	19	Yeasts	32, 94, 121
Seroepidemiologic studies	161		
Smoking	76		