
CONTENTS

Application of new serological (Major Membrane Protein II) Enzyme Linked Immunosorbent Assay for leprosy patients in Myanmar	121
<i>Khin Nwe Oo, Mi Mi Htwe, Khine Zar Win, Khin Thet Wai, Kyaw Myint & Kyaw Kyaw</i>	
Cloning of the hepatitis B surface antigen gene (2): amplification, restriction enzyme digestion and ligation of HBsAg gene and yeast plasmid pPIC9K	126
<i>Win Aung, Yeon Hee Kim, Hyunah Kim, Suk-Hoon Ha & Khin Pyone Kyi</i>	
Phytochemical analysis of Myanmar Green Tea: implications to antioxidant properties and health benefits	132
<i>Khin Tar Yar Myint, Thaw Zin, Khin Chit, Win Win Maw, Thandar Myint & Khin Myat Tun</i>	
Are they willing to provide adolescents reproductive health services?: Basic Health Staff's perspectives in Myanmar	138
<i>Myo Myo Mon, Saw Saw, Theingi Myint, Yin Thet Nu Oo, Wai Wai Myint, San San Aye & Pyone Thuzar Nge</i>	
Learning experiences of student nurses in clinical practice	144
<i>Tin Tun & Khin May Ohn</i>	
Plasma malondialdehyde, vitamin C and vitamin E levels of normal pregnant and preeclamptic pregnant women in West Yangon General Hospital	152
<i>Nilar Win, San Aye Kyi, Myo Win & Aye Aye Myint</i>	
Development of cytogenetic method for identification of <i>Anopheles culicifacies</i> species complex and its applications at malaria endemic areas of Myanmar	157
<i>Maung Maung Mya, Pe Than Htun, Sein Min & Sein Thaung</i>	
Sensitivity and specificity of parasite lactate dehydrogenase (pLDH) based rapid diagnostic test (SD Bioline malaria antigen test) in comparison with parasite density ...	164
<i>Khin Myo Aye, Myat Phone Kyaw, Tin Oo, Tin Oo, Kyin Hla Aye, Cho Cho Win & Cho Cho</i>	
Knowledge, attitude and practice on HIV/AIDS among the highway coach staff in Aung Mingalar Highway Coach Station	171
<i>Zayar Htun Tin, Kyu Kyu Than & Saw Saw</i>	

Socio-behavioral study on malaria prevention and control among forest related workers in hard-to-reach areas	178
<i>Moe Kyaw Myint, Khin Lin, Hla Soe Tint, Myitzu Tin Oung, Thi Thi Khaing, Khin Htet Zin, Aye Hnin Thwe & Marlar Aung</i>	
Success and challenges of Public-Private Mix DOTS initiatives in Myanmar : a process evaluation for partnership approach of non-governmental organizations	186
<i>Saw Saw, Thida, Thandar Lwin, Tin Mi Mi Khaing, Bo Myint, Khin Sandar Oo, Cho Cho Myint & Thandar Min</i>	
Short Report : Self-medication practices in a peri-urban area of Yangon City : a qualitative approach	194
<i>Thet Min Tun, Htein Lin & Kyaw Hlaing</i>	

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Application of new serological (Major Membrane Protein II) Enzyme Linked Immunosorbent Assay for leprosy patients in Myanmar

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As serodiagnosis is the easiest way of diagnosing a disease, the utility of (Major Membrane Protein II) MMP-II antigen in the serodiagnosis of leprosy was examined and compared with NTP-BSA ELISA test. It was carried out on the blood samples of new leprosy cases and their contacts from Nyaungdon Township, and on new adult pulmonary TB cases of Yangon TB Center and childhood TB cases of Yangon Children's Hospital and North Okkalapa General Hospital between November 2006 and December 2007. The sensitivity of the tests on detecting leprosy patients who had not been treated was 58/77 (75.32%) and 54/77 (70.1%) for MB patients using MMP-II and NTP-BSA ELISA tests, respectively. It was 48/64 (75.0%) and 40/64 (62.50%) for PB patients using MMP-II and NTP-BSA ELISA tests, respectively. The sensitivity of MMP-II ELISA test was higher than that of NTP-BSA ELISA test on both leprosy patients. The sensitivity of MMP-II ELISA test was also higher than that of NTP-BSA ELISA test on TB patients in our study. Therefore, our data indicate that MMP-II ELISA could be useful as a supporting serodiagnostic tool in diagnosis of leprosy and childhood tuberculosis.

INTRODUCTION

Leprosy represents a broad spectrum disease caused by *Mycobacterium leprae* with lepromatous leprosy at one pole and tuberculoid leprosy at another pole, depending on the clinical manifestation, which is an ultimate effect of the immunity of the host [1]. In all forms of the disease, *M. leprae* induces skin lesions and a chronic progressive peripheral nerve injury, to a lesser or greater extent, which leads to systemic deformity [2, 3]. Therefore, early detection of *M. leprae* infection is the key to avoiding deformities. The diagnosis of leprosy is based on microscopic detection of acid-fast bacteria (AFB) in skin smears or biopsies along with clinical and histo-

pathological evaluation. Acid-fast staining requires at least a thousand organisms per gram of tissue for reliable detection [4], resulting in an extremely low sensitivity especially for the tuberculoid form of the disease where AFB are rare or absent.

Serodiagnosis is generally accepted as the easiest way of diagnosing a disease. For leprosy, the only antigen currently used is phenolic glycolipid 1 (PGL-1), which is supposedly specific to *M. leprae* [5, 6]. Since the identification of PGL-1 in 1981 by Hunter and Brennan, a number of serological tools have been developed [7]. Simple assays such as the Serodia-Leptrae method, a dip-stick assay and lateral flow tests based on PGL-1 antigen have been used to detect leprosy patients in areas

where leprosy is endemic [8, 9]. However, these tests seem to be insufficient for detection of both multibacillary (MB) and paucibacillary (PB) patients as well as for early diagnosis.

Recently, real time PCR based methods have been developed [10], but the sensitivity of the test for clinical specimens is still problematic. In developing countries where leprosy is endemic, diagnosis still relies on clinical observations and easy inexpensive tests. Serodiagnosis is the easiest and tangible way of diagnosing a disease.

In Myanmar, leprosy elimination (Prevalence Rate $<1/10,000$) was achieved at national level at the end of January 2003. Until 2003, NCDR (New Case Detection Rate $>4/100,000$) was still high in some townships of Yangon, Ayeyawady, Bago, Mandalay, Magwe, Sagaing Divisions and Shan State [11].

To date, various antigens of *M. leprae* have been studied [12], but due to the lack of either specificity or sensitivity, their use had been limited. Major membrane protein II (MMP-II, ml2038c, gene name bfrA, also known as bacterioferritin) had been identified previously from the cell membrane fraction of *M. leprae*, as an antigenic molecule capable of activating both antigen-presenting cells and T cells [13, 14]. These findings prompted examination of the role of MMP-II as the role of humoral responses of patients.

As serodiagnosis is the easiest way of diagnosing a disease, the utility of MMP-II in the serodiagnosis of leprosy was examined. The percent positivity by an ELISA for anti MMP-II antibody was 82.4% for MB leprosy and the specificity of the test was 90.1%. For PB leprosy where cell-mediated immunity predominates, 39% showed positive results. These percentage values were significantly higher than those obtained by existing PGL-1 based methods suggesting that MMP-II antibody detection would facilitate the diagnosis of leprosy [15].

In the present study, the serological test using MMP-II as an antigen for serodiagnosis of leprosy was carried out and the sensitivity and specificity was compared with NTP-BSA (Natural Trisaccharide Phenyl-Propionyl-Bovine Serum Albumin) ELISA test. Both ELISA tests are sandwich types of ELISA.

MATERIALS AND METHODS

This study was a community and laboratory-based cross-sectional type of study and was conducted to compare the sensitivity and specificity of MMP-II with NTP-BSA ELISA on new MB and PB cases of leprosy and their contacts and tuberculosis cases. The blood samples were collected from new leprosy cases and their contacts of Nyaungdon Township, and new adult pulmonary TB cases of Yangon TB Center, childhood TB cases from the Yangon Children's Hospital and North Okkalapa General Hospital. The samples were collected between November 2006 and October 2007.

Sample collection

The blood samples were collected from new leprosy cases and their contacts from Nyaungdon Township, Ayeyawady Division. They were also collected from smear-positive and negative pulmonary tuberculosis cases attending the Yangon TB Center and childhood TB cases of YCH and NOGH. To get the community acceptability, the cases and subjects were informed that a research study of leprosy would be done. The objectives of the study were explained. Two millilitres of blood were taken after obtaining informed consent from each subject who is above 12 years of age. If the subject is less than 12 years of age, informed consent was taken from his/her parents or guardians. The blood was withdrawn using sterile needles and syringes. The clotted blood samples were transported under cold storage to the Bacteriology Research Division, Department of Medical Research (Lower Myanmar).

NTP-BSA (Natural Trisaccharide Phenyl-Propionyl–Bovine Serum Albumin) ELISA and MMP-II (Major Membrane Protein II) ELISA tests were carried out on these sera.

Leprosy examination

Leprosy examinations were performed by trained leprosy field workers of Nyaungdon Township. Leprosy was clinically diagnosed based on detailed skin examination, including testing for anesthesia and examination for enlargement of nerves and confirmed by the Regional Officer. Classification was based on the WHO system of lesions counting (paucibacillary PB patients=1-5 lesions, multibacillary MB patients=>5 lesions) [16].

Procedure for indirect ELISA

NTP-BSA (Natural Trisaccharide Phenyl-Propionyl–Bovine Serum Albumin) and MMP-II (Major Membrane Protein II) ELISA tests, both sandwich types, were carried out on the samples. The seropositives were identified from cut-off point of controls (apparently healthy blood donors of National Blood Bank, Yangon). The ELISA tests were carried out between December 2006 and November 2007. The ELISA microtitre plate (Fastec microplate U, Fujirebio Inc.) with 96 U-shaped wells was coated with 50µl of 1 µg/ml and 4 µg/ml concentrations of soluble antigens: NTP-BSA and MMP-II on each plate, respectively, and incubated at 37°C in moist chamber for 2 hours and then placed in 4°C for overnight. The coating antigens were diluted with carbonate-bicarbonate buffer to get final concentrations. The goat anti-human IgM (for NTP-BSA) and IgG (for MMP-II) conjugated to horse-radish-peroxidase (Dako, Denmark) were used for 2nd antibody reaction and ortho-phenylenediamine (Dako, Denmark) was used as the substrate. The optical density (OD) was measured by ELISA reader (Stat Fax 3200, USA) at wavelength of 492 nm within 15 minutes. Pooled sera from 10 multibacillary patients and 10 apparently healthy donors of the National Blood Bank, Yangon were used as positive and negative controls,

respectively. The cut-off values of ELISA tests were 0.015 for MMP-II and 0.07 for PGL-1 tests.

Study population

This study was carried out on 77 MB and 64 PB cases, their contacts and 80 blood donors. It was also studied on sputum smear-positive and negative adult pulmonary and childhood tuberculosis cases.

Informed consent

All eligible subjects were invited verbally for the study and procedure on taking blood samples was explained. Informed consent was requested from each adult. For children, less than 12 years of age, consents were obtained from their parents or guardians.

Study subjects

The diagnosis of leprosy was carried out according to the WHO guidelines and was performed by trained leprosy field workers from Nyaungdon Township. All suspected cases were examined by the Leprosy Regional Officer, Ayeyawady Division and were confirmed by the Central Special Skin Clinic, Yangon. All TB cases were diagnosed by specialists from Yangon TB Center and pediatricians and confirmed by radiologists.

Data analysis

The sensitivity and specificity of MMP-II ELISA test was calculated and compared with NTP-BSA ELISA test using ROC curve (Area under the curve) analysis.

RESULTS

The 2 ELISA tests were carried out on 77 multibacillary and 64 paucibacillary cases and 80 blood donors. The specificity of the tests, as calculated by using results of sera from 80 blood donors, was 69/80 (86.25%) for both MMP-II and NTP-BSA ELISA tests. The sensitivity of the tests in detecting leprosy patients who had not been treated was 58/77 (75.32%) and 54/77 (70.1%) for

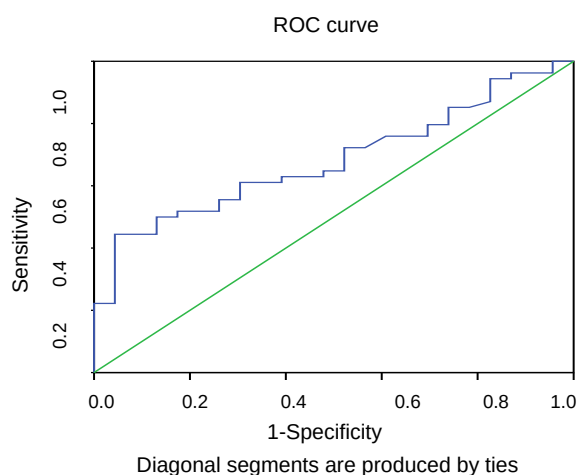
MB patients using MMP-II and NTP-BSA ELISA tests, respectively. It was 48/64 (75%) and 40/64 (62.5%) for PB patients using MMP-II and NTP-BSA ELISA tests, respectively. The sensitivity of MMP-II ELISA test was higher than that of NTP-BSA ELISA test in both leprosy patients (Table 1).

Table 1. ELISA results of leprosy and non-leprosy patients in Myanmar

No. of cases	MMP-II (%)	PGL-1 (%)
<i>Patient</i> (141)	106 (75.2)	94 (66.7)
<i>Normal</i> (80)	11 (13.8)	11 (13.8)
<i>Contacts</i> (472)		
Contact to MB (317)	281 (88.60)	74 (23.3)
Contact to PB (155)	123 (79.4)	36 (23.20)
Total	404 (85.6)	110 (23.3)
<i>TB patients</i> (196)	27 (55.10)	0 (0)
Smear + TB (49)		
Smear - TB (52)	7 (13.5)	0 (0)
Child NOGH (50)	18 (36)	4 (8.00)
Child YCH (45)	37 (82.2)	4 (8.9)
Total	89 (45.4)	8 (4.1)

Cut-off value: >0.015 for MMP-II
>0.07 for PGL-1

By using ROC curve analysis; for MMP-II serodiagnostic test, area under the ROC curve was 0.855; standard error, 0.028; 95% CI, (0.795 to 0.902); and $p=0.0001$.

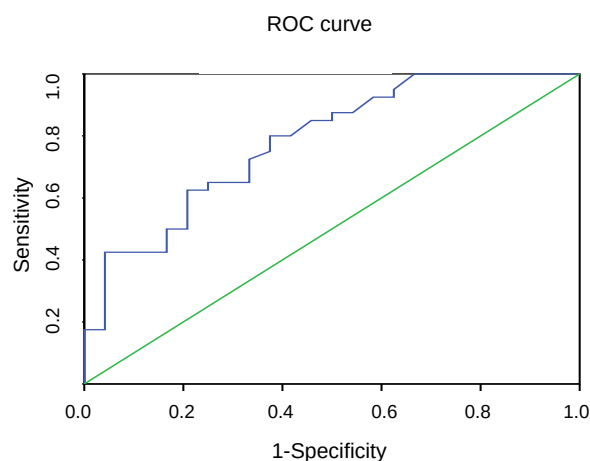


Area under the curve

Test result variable(s): mmp2 value

Area	Std. error (a)	Asymptotic sig.(b)	Asymptotic 95% confidence interval	
			Lower bound	Upper bound
.682	.061	.012	.564	.801

Fig. 1. ROC curve of multibacillary cases



Area under the curve

Test result variable(s): mmp2 value

Area	Std. error (a)	Asymptotic sig.(b)	Asymptotic 95% confidence interval	
			Lower bound	Upper bound
.782	.059	.000	.666	.898

Fig. 2. ROC curve of paucibacillary cases

For NTP-BSA ELISA test, area under the ROC curve was 0.872; standard error, 0.026; 95% CI, (0.815 to 0.917); and $p=0.0001$. The sensitivity of MMP-II ELISA test was higher than that of NTP-BSA ELISA test in TB patients of this study (Fig. 1& 2).

DISCUSSION

In the study involving Vietnamese leprosy patients, there is a significant difference between MMP-II ELISA and PGL-1 ELISA in detecting both MB and PB leprosy. The positivity rate of anti-MMP-II antibody for MB leprosy was approximately 85% and that for PB leprosy was 48%, significantly higher than the titers obtained by PGL-1 ELISA (57% and 20%, respectively) [14]. Also in our study, the sensitivity of MMP-II ELISA test was higher than that of NTP-BSA ELISA test in both leprosy patients.

The difference in sensitivity between PGL-1 ELISA and MMP-II ELISA may be due to differences in the biochemical features of the antigens. PGL-1 is a glycolipid component, and as such, it might be retained in some infected cells for a longtime after

the initial exposure [17]. Therefore, the usefulness of PGL-1 based tests for early diagnosis is limited. MMP-II is a protein antigen and is considered to be one of the immunodominant antigens of *M. leprae* [14]. Therefore, in individuals who had been exposed to *M. leprae* but did not have leprosy, antigen presenting cells expressing MMP-II might feasibly be eliminated from the body by immune cells such as cytotoxic T lymphocytes and thus lack the ability to produce anti MMP-II antibodies through antigen presenting cell dependent mechanisms.

Since the MMP-II protein is conserved in response to exposure to other mycobacteria, such as *M. tuberculosis* and *M. avium*, the sensitivity of MMP-II ELISA test was slightly higher than that of NTP-BSA ELISA test on TB patients in our study. Therefore, our data indicate that MMP-II ELISA could be useful as a supporting serodiagnostic tool for leprosy and childhood tuberculosis.

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This study was granted by the International Medical Center of Japan. The coating antigen of MMP-II ELISA test was a gift from Professor M Kai, National Institute of Infectious Diseases, Tokyo, Japan.

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Cloning of the hepatitis B surface antigen gene (2): amplification, restriction enzyme digestion and ligation of HBsAg gene and yeast plasmid pPIC9K

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DNA cloning involves separating a specific gene or DNA segment from a larger chromosome, attaching it to a small molecule of carrier DNA, and then replicating this modified DNA thousands or millions of times through both the increase in cell number and the creation of multiple copies of the cloned DNA in each cell. The result is selective amplification of a particular gene or DNA segment. In a recent preliminary study, extraction and purification of the HBsAg gene from genomic DNA of *H. polymorpha* transformant cell, followed by ligation with pGEM-T vector and transformation into the competent *E. coli* DH5 alpha cells were successfully carried out. In this study, the HBsAg gene containing plasmid pGEM-T was amplified by using specific primers with restriction enzyme (RE) digestable nucleotide sequences followed by RE digestion with *EcoR* I and *Not* I to obtain sticky ends for an effective ligation. At the same time, the yeast expression plasmid, pPIC9K was digested with same RE to have sticky ends compatible with those of HBsAg gene containing plasmid. Then, these DNA segments with compatible nucleotide sequences were ligated, followed by successful transformation into the competent *E. coli* cells. The results from each and every step were confirmed by direct PCR identification, RE analysis followed by agarose gel electrophoresis determination and DNA sequencing analysis. These initial steps are important and essential for further transformation of the recombinant HBsAg gene into the host *Pischia pastoris* yeast cell which can be used as a more productive organism for production of recombinant hepatitis B vaccine in the near future.

INTRODUCTION

Recombinant DNA technology, which is also called gene cloning or molecular cloning, allows the recombinant of DNA molecules that would never or very rarely occur in nature, leading to transfer of genetic information from one organism to another. DNA cloning involves separating a specific gene or DNA segment from a larger chromosome, attaching it to a small molecule of carrier DNA, and then replicating this modified DNA thousands or millions of times through both the increase in cell number and the creation of multiple copies

of the cloned DNA in each cell. The result is selective amplification of a particular gene or DNA segment [1].

For development of recombinant hepatitis B (HB) vaccines, HB virus recovered from plasma of a hepatitis B carrier has been used to prepare viral DNA that has been cloned in *E. coli*. Then the gene coding for HBsAg has been amplified and isolated. Prior to the transformation of the gene of interest into the yeast cells, plasmid construction has to be performed. Finally, the HBsAg gene has been inserted into yeast by means of appropriate expression yeast vectors. Purified HBsAg proteins expressed from

transformed yeast cells containing it have been formulated into HB vaccines and shown to induce protective antibody response in human [2].

The majority of currently available recombinant hepatitis B vaccines in the market have been produced by using appropriate expression plasmids in yeast cells. Since 1986 to date, *Saccharomyces cerevisiae* and *Hansenula polymorpha* have been used as host systems for commercial production of recombinant HB vaccines. Recently, an increasing number of publications attests to the favorable properties of methylotrophic yeasts as hosts for heterologous gene expression. Among them, *Pichia pastoris* yeast cell is emerging as the most significant industrial application for production of heterologous recombinant proteins [3, 4, 5, 6].

In a recent preliminary study, extraction and purification of the HBsAg gene from genomic DNA of *H. polymorpha* transformant cell, followed by ligation with pGEM-T and transformation into the competent *E. coli* DH5 alpha cells were successfully carried out [7]. In this study, attempts were made to extract the HBsAg gene containing plasmid from transformed *E. coli* cells followed by amplification, restriction enzyme digestion, ligation of the HBsAg gene with the yeast plasmid and transformation into competent *E. coli* cells for further transformation into final host, *Pichia pastoris* yeast cells with an aim to produce recombinant HB vaccine in the near future.

MATERIALS AND METHODS

HBsAg containing plasmid

The HBsAg gene containing pGEM-T plasmid of ~3700 base pairs was obtained from the transformant *E. coli* DH5 alpha cells recently prepared in the laboratory of Research and Development Centre of Pharmaceuticals, Institute of Science and Technology, CJ Corporation, Ichon City, Republic of Korea [7].

Host strains

The *E. coli* DH5 alpha strain, grown in Luria-Bertani (LB) media supplemented with ampicillin and stored at -20°C was used in cloning technique for plasmid amplification and transformation.

Expression vector

The closed circular vector, pPIC9K of 9276 base pairs (bp), containing the kanamycin resistance gene for *in vivo* screening of multicopy inserts, was commercially available from the Promega Co., USA (Fig.1).

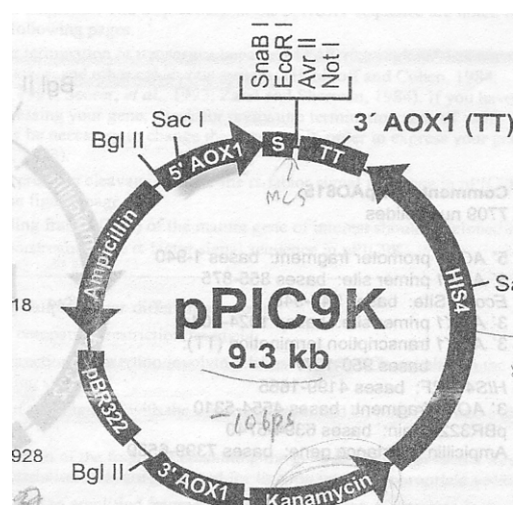


Fig. 1. Physical map of plasmid pPIC9K (976 bp)

Because of the presence of kanamycin (Kan) gene that confers resistance to geneticin in *Pichia*, only cloned gene integrated in this vector can survive on media with geneticin and it can be used as a tool to detect transformants that harbours multicopies of the gene.

Ligation of the HBsAg gene with yeast plasmid vector

Preparation of the HBsAg gene for ligation

A single colony of *E. coli* transformant containing the HBsAg gene in pGEM-T vector (non-linearized) was inoculated into LB media with ampicillin, followed by extraction of the closed circular recombinant plasmid using the AccuPrep plasmid extraction kit (Bioneer). The recombinant

gene was amplified by PCR using specific primers designed with the desired RE digestible nucleotide sequences :9S (5'-AAA GAA TTC ATG GAG AAC ATC ACA TCA GGA-3') and 35 A (5'-AAA GCG GCC GCT TAA ATG TAT ACC CAA AGA CA-3'). The amplified target recombinant DNA with specific nucleotide sequences was purified by using the PCR purification test kit from QIAGEN, USA [8]. The purified DNA segment was digested at specific nucleotide sequences by restriction enzymes (REs) *EcoR* I and *Not* I from Roche. The complete digestion was confirmed by determination on agarose gel electrophoresis. The specific and linearized RE digested DNA segment of interest with sticky ends was then extracted and purified by using the gel extraction kit (QIAGEN, USA), confirmed by agarose gel electrophoresis and quantified by spectrophotometric determination at the wavelength of 260 nm.

Preparation of yeast plasmid for ligation

A single colony of *E. coli* transformant containing yeast expression vector pPIC9K, which was commercially available from the Invitrogen Life Technologies, was inoculated into the LB media with ampicillin and extracted by using the method of the alkaline lysis with Sodium Dodecyl Sulfate (SDS) followed by RNase treatment [9]. These extracted yeast plasmids were then purified by the Phenol and Chloroform method [10].

The purified closed and circular plasmids containing the desired nucleotide sequences were digested by same RE, used in the treatment of the DNA segment of interest i.e *EcoR* I and *Not* I to obtain the linearized plasmid DNA with sticky ends, and the complete digestion was confirmed by agarose gel electrophoresis. The linearized plasmids were extracted by the gel extraction kit (QIAGEN, USA), followed by treatment with alkaline phosphatase for prevention of self ligation. This non-circularized plasmid was further purified by using the gel extraction kit (QIAGEN, USA)

and confirmed by agarose gel electrophoresis and quantified by electrophoretic determination .

Ligation of the HBsAg gene and yeast plasmid pPIC9k

The linearized HBsAg gene and plasmid, both with compatible sticky ends were incubated in the 16°C water bath for 6 hours followed by incubation at 4 °C over night by using the T4 DNA ligase from Promega, USA for specific DNA ligation [11].

Transformation of the recombinant HBsAg gene containing plasmid into E. coli cells

The above linearized recombinant DNA segment pGMT-T plasmid containing the HBsAg gene was transformed into the competent *E. coli* DH5 alpha cells which had already been prepared in the laboratory, by using the heat-shock procedure i.e. 42 °C for exactly 90 seconds without shaking [12]. The transformant containing non-linearized recombinant plasmid was identified as appearance of white colonies in a culture plate containing LB medium with ampicillin. A single colony of *E. coli* transformant containing plasmid-insert DNA construct was inoculated into LB media with ampicillin, followed by extraction using the alkaline lysis with SDS method and further purified by the Phenol-Chloroform method. The extracted and purified recombinant DNA was confirmed by RE analysis with *EcoR* I and *Not* I, followed by agarose gel electrophoresis. The presence of the HBsAg structural gene was also confirmed by direct colony PCR (using specific primers 9S and 35A, annealing temperature of 50°C, repeated cycles of 30), followed by agarose gel electrophoresis determination and DNA sequencing analysis by using the ABI Prism 3100 Genetic Analyzer [13].

RESULTS

Fig. 2 represents the electrophoretic determination of the HBsAg DNA segment and the plasmid pPEM-T transformed into *E. coli* cell after restriction enzyme digestion with

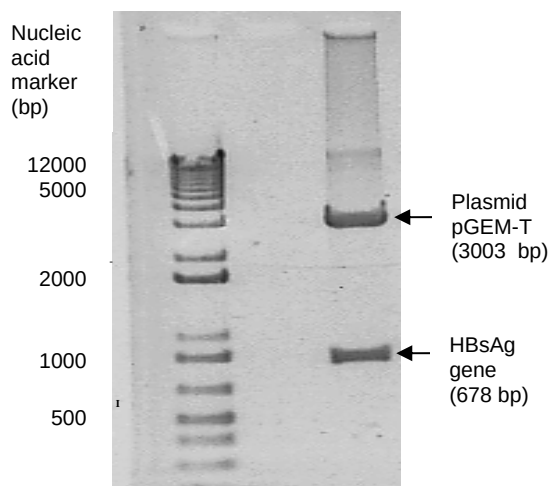


Fig. 2. Electrophoretic determination of HBsAg gene and the plasmid pGEM-T after RE digestion with *EcoR* I and *Not* I

EcoR I and *Not* I. Two distinct DNA bands of the HBsAg gene and pGEM-T vector were detected at levels of ~700 bp and ~3000 bp, respectively, indicating the complete digestion of the plasmid-insert DNA construct by the specific enzyme. Electrophoretic identification of extracted and purified plasmid pPIC9K, before and after digestion with *EcoR* I and *Not* I is shown in Fig. 3.

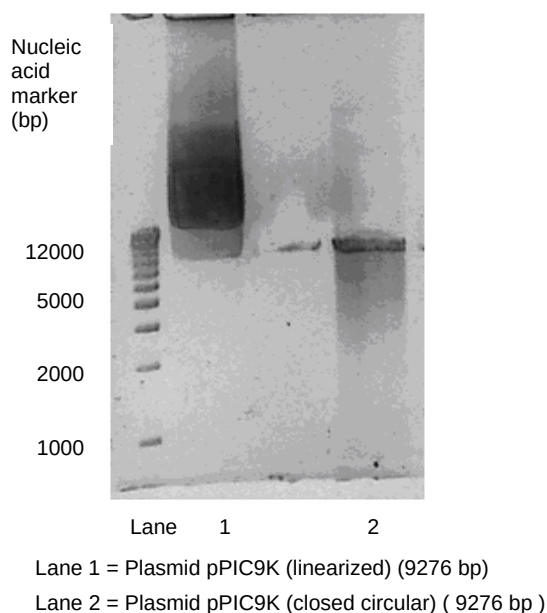


Fig. 3. Electrophoretic identification of the extracted and purified plasmid pPIC9K before and after RE digestion with *EcoR* I and *Not* I

Both DNA bands were observed at the level of ~9000 bp but the electrophoretic mobility of linearized plasmid was found to be slightly faster than that of the close and circular plasmid. The linearized plasmid with sticky ends was used for further recombination with compatible DNA segment.

Electrophoretic identification of non-circularized plasmid pPIC9K with desired nucleotide sequences after digestion with alkaline phosphatase is shown in Fig. 4. A distinct DNA band was detected at the level of ~10,000 base pairs, indicating the efficient transformation of non-circularized pPIC9K into *E. coli* cells.

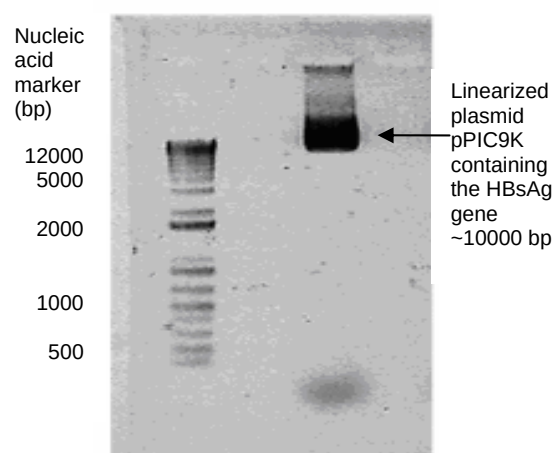


Fig. 4. Electrophoretic identification of linearized plasmid pPIC9K with desired nucleotide sequences after digestion with alkaline phosphatase

Fig. 5 represents the electrophoretic identification of the plasmid-insert DNA construct before and after digestion with *EcoR* I and *Not* I. Only a single band was observed at the level of ~10,000 base pairs before RE digestion whereas two distinct bands were observed at the levels of ~10,000 bp and ~700 bp, thus indicating the presence and complete digestion of the cloned DNA.

Fig. 6 demonstrates agarose gel electrophoresis pattern of the HBsAg gene after the direct colony PCR identification of the HBsAg DNA segment cloned with plasmid pPIC9K vector in the *E. coli* transformant. The nucleotide sequence of the purified

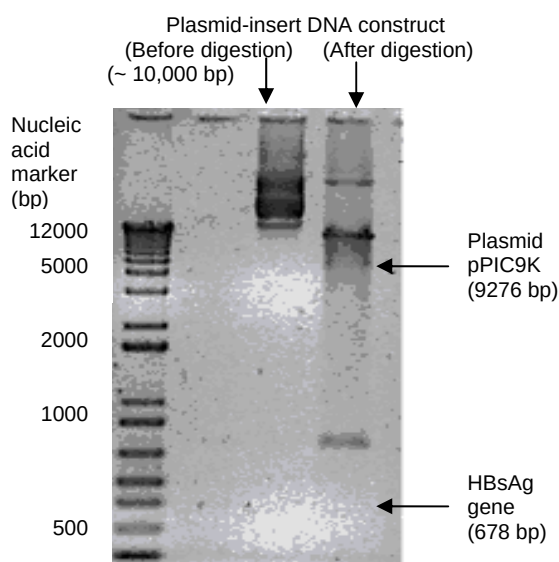


Fig. 5 . Electrophoretic identification of the plasmid-insert DNA construct before and after RE digestion with *EcoR* I and *Not* I

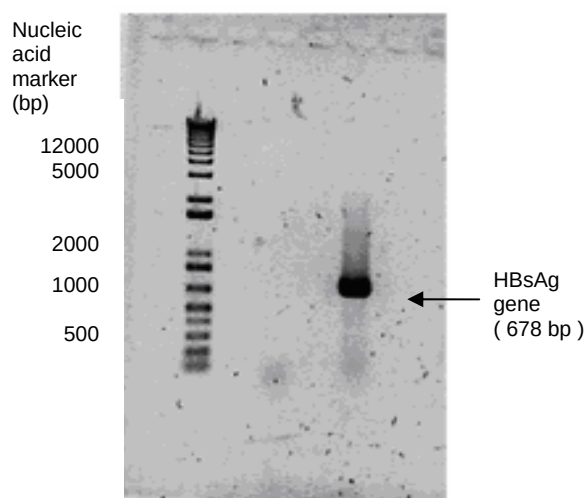


Fig. 6. Direct colony PCR identification of HBsAg gene integrated in plasmid pPIC 9K in *E. coli* transformant

HBsAg segment, extracted and purified from transformant *E. coli* cell was analyzed by the ABI Prism 3100 Genetic Analyzer. It was found that the nucleotide sequences obtained were totally identical to the theoretical sequences of the HBsAg gene, indicating the successful transformation of the HBsAg gene into competent *E. coli* cell after ligation with pPIC9K.

DISCUSSION

Recombinant DNA is an altered DNA due to the insertion of a sequence of deoxy-ribonucleotide not present in an existing molecule of DNA by enzymatic or non-enzymatic means. These DNA fragments, obtained by restriction endonuclease treatment of chromosome, can be cloned. In order to obtain a large amount of a particular fragment of DNA, cloning vehicle plasmid is required. Plasmid is a small extra-chromosomal circular molecule of DNA that replicates independently of the host DNA. Virtually all bacteria genera have plasmids. Some plasmids carry information for their own transfer from one cell to another; others encode resistance to antibiotics; others carry specific sets of genes for the utilization of unusual metabolites; and some have no apparent functional coding genes. Plasmids can range in size from less than 1 kb to more than 500 kb. Each plasmid has a sequence that functions as an origin of DNA replication; without this site, it cannot replicate in a host cell. Replication and amplification of the plasmid containing a gene of interest can be performed in microorganisms. However, some plasmids, because of the specificity of their origin of replication, can only replicate in one specific species. They can be introduced into the bacterial cells by a process called transformation [14].

In this study, the HBsAg gene containing plasmid pGEM-T was amplified by using specific primers with RE digestible nucleotide sequences followed by RE digestion with *EcoR* I and *Not* I to obtain a sticky end for an efficient ligation. At the same time, the yeast expression plasmid, pPIC9K was digested with same RE to have sticky ends compatible with those of HBsAg gene containing plasmid. Then, these DNA segments with compatible nucleotide sequences were ligated, followed by successful transformation into the competent *E. coli* cells. The results obtained from each step were confirmed by direct PCR identification, RE analysis followed by agarose

gel electrophoresis determination and DNA sequencing analysis. Since the yeast plasmid amplification can never be achieved in yeast cells, an initial plasmid construction was performed in *E. coli* cells before transferring into the ultimate host, *Pichia pastoris* yeast cells.

Our study described the effective amplification of the HBsAg gene, complete restriction enzyme digestion, ligation of the HBsAg gene with yeast plasmid, and successful transformation of the plasmid-insert DNA construct into the competent *E. coli* cells. These steps are important and essential for further transformation of the recombinant HBsAg gene into the ultimate host, *Pischia pastoris* yeast cell which can be used for production of recombinant hepatitis B vaccine in the near future. Further research works on transformation of the recombinant HBsAg gene into the *Pichia pastoris* yeast cells are in progress at the laboratory of Research and Development Centre of Pharmaceuticals, Institute of Science and Technology, CJ Corporation, Ichon City, Republic of Korea.

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Phytochemical analysis of Myanmar Green Tea: implications to antioxidant properties and health benefits

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Many people around the world drink green tea for its reputed health benefits, which are believed to be attributed to the presence of polyphenols. Polyphenols contained in tea are classified as catechins, which are chemicals with potent antioxidant properties and thus, act as scavengers of free radicals. This antioxidant property of green tea is dependent on the gentle steaming method which prevents oxidation and thus preserving the polyphenols in its original form. Myanmar Green Tea (Nara Organic Green Tea; Kachin Special Group) and Plain Tea (Htoo Super Plain Tea), commercially available in the market, were subjected to qualitative and quantitative analysis of its constituents including alkaloids such as caffeine, catechin containing polyphenols, and tannins. The results showed that Myanmar Green Tea has a higher percentage of polyphenols than Plain Tea, thus supporting the preservation of antioxidant properties and its health benefits. Presence of alkaloids including caffeine and related compounds is responsible for the stimulant effect of both plain tea and green tea. The study supported the importance of the processing methods in making tea if the beneficial effects are to be preserved.

INTRODUCTION

Millions of people around the world continue to use herbal remedies because they believe in them [1]. Among these remedies are various forms of tea, scientifically known as *Camellia sinensis*, of which the leaves are widely consumed, both as traditional food and medicine. Green tea, which is highly reputed throughout the world for its health benefits, is believed to be attributed to the presence of antioxidant properties [2]. In Myanmar, there are 3 varieties of tea; plain tea, black tea and fermented tea (La-phet). Most people in Myanmar believed green tea and plain tea to be the same. However, in reality, the processing methods of these two forms are different. In Myanmar, of the well known brands, the Nara Organic Green Tea is processed by modernized stream distillation method while the "Htoo" Super Plain Tea is

processed by the traditional method of drying, either by sunlight and/or by oven heating. Thus, it becomes interesting to know which of the methods used in Myanmar, is the best to preserve the antioxidant properties and the reputed health benefits, through research into the phytochemical constituents and tests for antioxidant activity in these two brands.

Currently, antioxidants are believed to help protect the body from free radical damage. Long-term use of vitamins and nutrients to prevent aging and diseases has been known and recent evidence has pointed out that such substances found in natural sources like fruits, leaves and vegetables rich in plant flavonoids are excellent antioxidants [eg. ascorbic acid from Zee-Phyu-Thee, retinoids and carotenoid from carrots, allicin in garlic and polyphenols (catechins) in tea leaves]. Thus, research to preserve the antioxidant properties of natural food and

food products, in preventing aging, diseases of sedentary living such as atherosclerosis, hypertension, cancer, diabetes and obesity, and even in smoking and environmental pollution has become an interesting topic of the 21st century [3-13].

Objectives

- To determine the phytochemical constituents in the tea leaves subjected to different processing methods during their preparation.
- To compare the free radical scavenging activity of Nara Green Tea and Htoo Super Plain Tea.

MATERIALS AND METHODS

Nara Green Tea consists of young tea leaves prepared by steam distillation process and Htoo Super Plain Tea consists of mature tea leaves prepared by heating and drying. Both samples were purchased locally from the market. Qualitative comparison of phytochemical constituents in Nara Green Tea and Htoo Super Plain Tea was done by the method of Harborne, 1984 [14] and quantitative measurement of the active compound of alkaloid and polyphenols was done by Unani Formulation, 1987 [15].

Total alkaloid

Ten grams of sample from each brand were accurately weighed, and extracted with 0.5N H₂SO₄ before being filtered. The filtrate was mixed with 30 ml chloroform and shaken for 30 minutes. The lower chloroform layer was collected using a separating funnel and the remaining upper acid layer made alkaline by adding an excess of dilute NH₄OH and shaken for 20 minutes. After standing for 10 min, the chloroform layer was separated and later removed using a Rotatory Evaporator. The remaining dried residue was dehumidified in a vacuum desiccator until a constant weight was obtained and the final weight was taken as total alkaloid. This separated alkaloid compound, which should represent the caffeine content, was checked by standard

color test and Thin Layer Chromatography (TLC), and further quantitated by UV-1601 spectrophotometer.

Total phenolics

Ten grams of sample from each brand were extracted with water and filtered. The filtrate was shaken with petroleum ether (PE) for 30 minutes. The lower PE layer was discarded and the upper aqueous layer was precipitated by saturated solution of lead acetate. The precipitate was digested for 5 minutes in a water bath and filtered. The dried residue was suspended in alcohol, in slightly warmed water bath (38°C), after which the clear alcoholic solution obtained was further concentrated using a Rotatory Evaporator. The dried residue was dehumidified in a vacuum desiccator until constant weight was obtained. The obtained polyphenolic compounds were separated by TLC and the catechin (epigallocatechin gallate) was checked by color test and UV-1601 spectrophotometer.

Measurement of DPPH radical scavenging activity by spectrophotometric method [16, 17]

DPPH (1,1-Diphenyl-2-picryl-hydrazyl) stock solution, 60 µM (DPPH 2.36 mg in 100 ml of 95% ethanol), was freshly prepared and stored in brown colored volumetric flask at +5°C (no longer than 24 hrs) before use. Serial dilutions of the watery extract of test samples in concentrations of 0.625, 1.25, 2.5, 5 and 10 µg/ml were prepared in a 50% ethanolic solution. The test sample was also prepared by mixing 2 ml of 60 µM DPPH solution and 2 ml of watery extract (test solution) vigorously by a vortex mixer. Blank solution was prepared by mixing equal volume of test sample solution and 50% ethanolic solution. All solutions were allowed to stand at room temperature for 30 minutes after which measurement of absorbance was done at 517 nm using a UV-1601 Shimadzu Spectrophotometer. Absorbance measurements were done in triplicate and calculated to obtain the % inhibition using the following formula:

% inhibition = $\frac{\text{DPPH alone} - (\text{sample-blank})}{\text{DPPH alone}} \times 100$
 % inhibition = Percent inhibition of test sample
 DPPH alone = Absorbance of control solution
 Sample = Absorbance of test sample solution
 Blank = Absorbance of blank solution

RESULTS

The findings indicated that the contents of alkaloid compound, polyphenol, glycoside and steroids were higher in Nara Green Tea than in Htoo Plain Tea. Detail results are shown in Table 1.

Table 1. Comparison of phytochemical constituents of Nara Green Tea and Htoo Super Plain Tea

Phyto-test	Reagent	Observed (color)	Results	
			Nara	Htoo
Alkaloid	Dragendroff's reagent	Orange ppt	+	+
Glycoside	NaOH solution	Yellow	++	+
Flavonoid	conc: HCl/Mg	Red	+	+
Polyphenol	10% FeCl ₂ sols:	Blue	+	+
Steroid	Acetic anhydride and conc: H ₂ SO ₄	Green blue	++	+
Terpene	Acetic anhydride and conc: H ₂ SO ₄	Pale yellow	-	-
Tannin	1% FeCl ₂ soln:	Yellowish brown	+	+
Reducing sugar	Fehling soln:	Brick red	+	++
CN glycoside	Picric paper	Brown	-	-
(+) detected			(-) not detected	

Total alkaloid content was higher in Nara Green Tea than Htoo Plain Tea (1.24% and 1.12%, respectively). Caffeine content was slightly higher in Nara Green Tea (4.8%) than in Htoo Plain Tea (3.2%) and the presence of caffeine was confirmed by TLC and UV spectrophotometer, showing a definite spectrum with a peak at 272.5 nm, detected in 0.1N HCl solution [14] (Fig.1).

The content of total phenolic compounds was higher in Nara Green Tea than in Htoo Plain Tea (3.6% and 2.6%, respectively). Catechin compound was detected in total phenolic by UV spectrophotometer, showing

a definite spectrum with a peak at 281.0 nm in methanol solvent (Fig. 1).

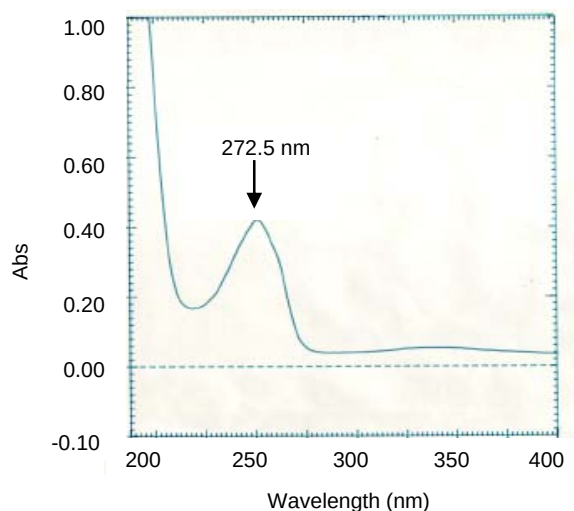
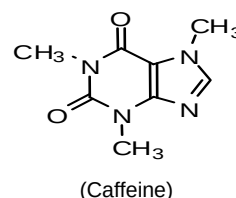
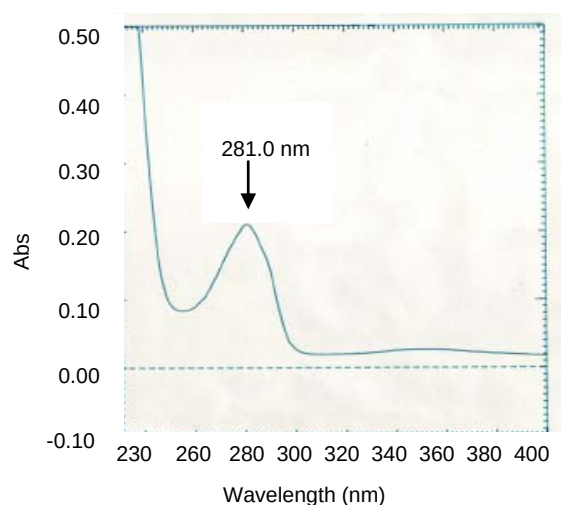
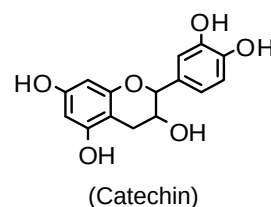


Fig. 1. UV-spectrum of caffeine and catechin compound in Nara Green Tea

Determination of radical scavenging activity of DPPH method is based on the change in absorbance of watery extracts solution in

various concentrations. Determination of absorbance values was carried out at wavelength 517 nm using spectrophotometer. Decrease in absorbance indicates increase in radical scavenging activity.

Free radical scavenging activity of watery extracts, usually expressed in term of % inhibition, is shown in Table 2.

Table 2 . Inhibition % of oxidation of watery extracts from tea leaves

No.	Extracts conc: (µg/ml)	% inhibition				
		0.625 µg/ml	1.25 µg/ml	2.5 µg/ml	5.0 µg/ml	10 µg/ml
1	Watery extract of Htoo plain tea		59.8	62.5	75.5	94.0
2	Watery extract of Nara plain tea		58.5	80.4	82.6	85.9
3	Ascorbic acid	69	83.2	94.6	98.9	99.5

From these results, it was also observed that increase in concentration showed to increase % inhibition, i.e, increase free radical scavenging activity.

DISCUSSION

Oxygen, the molecule of life for all aerobic creatures, undergoes chemical reactions with carbohydrate molecules, in the blood and the living cells, with the resultant generation of energy molecules such as ATP (adenosine triphosphate). During such process, undesirable side products known as free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are also generated regularly, both during normal organ function or excessive oxidative stress. The reactive species superoxide ($O_2^\bullet$), hydroxyl-radical ($^\bullet OH$), nitrogen oxide ($NO^\bullet$) and hypochlorous acid (HOCL) are all products of normal human metabolic pathway, which can exert harmful effects under certain conditions [18,19].

Over and above, environmental pollution, radiation, cigarette smoke and herbicides can form harmful free radicals that damage

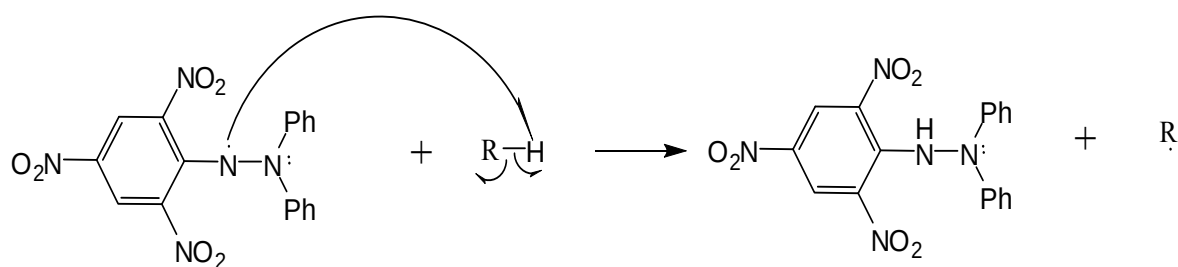
protein, DNA and other essential molecules. These gradual accumulations of proteins and DNA within the cells are now known to cause aging as well as diseases such as cancer and cardiovascular diseases [18].

Antioxidants are substances that scavenge free radicals damaging compounds in the body that alter cell membranes, tamper DNA (genetic material) and even cause cell death. Free radicals occur naturally in the body when subjected to oxidative stress, but environmental toxins including ultraviolet light, radiation, cigarette smoke, and air pollution can also increase the number of damaging particles. Free radicals are believed to contribute to the aging process as well as the development of cancer and cardiovascular diseases. It has been reported that polyphenols inhibit the growth of cancer cells, and may even kill them without harming the normal tissues [11, 18, 19].

Green tea is prepared from unfermented tea leaves and contains phenolic acid, polyphenols whose antioxidant effects has been known to be greater than Vitamin C. It has been reported that consumption of three cups of green tea per day (3 g soluble components, or 240 to 320 gm polyphenols) or 300 to 400 mg per day of standardized green tea extract (extracts should contain 80% total polyphenols and 55% epigallocatechin) is recommended for healthy living [19, 20].

Polyphenols in green tea are classified as catechins and involve 6 main compounds; catechin, gallic acid, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate (EGCG). This EGCG is considered to be the most active component in green tea, and thus, serves as a standard parameter determining the antioxidant property of a compound, and its presence is seen in green tea in the present study.

Commonly used assays for measurements of radical scavenger activity are as follows: Conjugated Diene Assay, Lipid Peroxide (PD) Assay, Thiobarbituric Acid Reactive Substances (TBARS), Carotene Linoleic



Acid System, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay, and Cyclic Voltammetry (CV). The present study utilized the measurement of radical scavenging activity of DPPH, a stable free radical, using the spectrophotometric method [17].

The principle of DPPH method is that, in the presence of a stable free radical (DPPH), an antioxidant donates a hydrogen atom to quench the stable free radical. Due to the ethanolic component, DPPH solution is violet and at 517 nm, which changes upon neutralization by free radicals from violet to pale yellow. Spectroscopic measuring of absorbance at 517 nm allows the determination of DPPH proportion being neutralized by the test substance, the decolouration of the initial color being proportional to the anti-radicalizing (antioxidant) power of test substances by Thin Layer Chromatographic method. This method can be applied either when the antioxidant in its pure form or in a mixture.

From these results, it was found that the absorbance decreases with increase in concentration (i.e., increase in concentration increase the radical scavenging activity).

The findings in the present study indicated that the polyphenols present in the tea leaves may differ depending on the processing methods used in preparing them. The difference can be due to the some polyphenols being destroyed (oxidized) to some extent during the process of drying in sunlight and/or heating in the oven. Thus, in spite of the use of same tea leaves, the polyphenol content was lower in the Htoo Plain Tea when compared to Nara Green Tea which is made from young tea leaves which are processed by gentle steaming.

This finding supported the importance of steam processing of green tea which prevents the EGCG compound from being oxidized.

Conclusion

The present study showed that the antioxidant activity is higher in Nara Organic Green Tea than Htoo Super Plain Tea. However, the difference in the antioxidant activity of same tea leaf used is due to different processing methods of the two brands. The antioxidant property of polyphenol from Nara Organic Green Tea is dependent on the gentle steaming method which preserves polyphenol in its original form. Therefore, destruction of the active compound is the result of difference in the health benefits in taking tea [20, 21].

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**Are they willing to provide adolescents reproductive health services?:
Basic Health Staff's perspectives in Myanmar**

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This study aimed to inquire the health providers' knowledge, perception and problems encountered in providing Adolescent Reproductive Health (ARH) services. A cross-sectional study employing both quantitative and qualitative approaches was conducted in two townships where ARH corners have been launched. All Basic Health Staff (BHS) were requested to answer a pre-tested, structured questionnaire. Four focus group discussions, 8 in-depth interviews and 4 key informant interviews were conducted. Duration of total service of BHS varied from less than one year to 40 years (median-7 years). About 67% of BHS got ARH training and 44%, 56%, 59% and 85% had high level of knowledge on reproductive physiology, adolescent pregnancy, contraception and HIV/AIDS issues, respectively. Adolescent child birth and abortions were mentioned as common ARH problems within their community. More than half agreed to provide Reproductive Health (RH) information to the adolescents hoping that it could prevent the ARH problems. However, some worried that providing RH knowledge might lead adolescents to promiscuity. About half stated that it should not be informed to adolescents under 15 years of age. Most providers were reluctant to give contraceptive knowledge to unmarried adolescents. Seventy-one percent had experience of providing ARH services of which 32% encountered some problems such as difficulty in recruiting adolescents, reluctance of adolescents to listen RH talks and unwillingness of parents, teachers and village leaders. Providers should be encouraged to impart reproductive health information to all adolescents regardless of the age and marital status.

INTRODUCTION

According to World Health Organization, all persons between the age of 10 to 19 are adolescents [1]. In Myanmar, reproductive health programme defines adolescents to include youth and cover the age range from 10 to 24 years old [2]. This represents 29% of the total population (CSO-2002). As they have to pass through physical changes related to human reproduction, they need information and opportunities to discuss sexuality in a safe and open way [3]. Thus the provision of reproductive health services

to adolescents is crucial. Along with the introduction of the reproductive health programme, health of young people is being considered as one of the priority areas to be focused nationally. A number of programmes that address adolescents' knowledge of sexual and reproductive health have been implemented in recent years, including life skills and peer education programmes [4]. One of the programmes that implemented recently by DOH with the support of UNFPA was launching of ARH corners in the UNFPA project townships.

Providers' perception to give quality services is essential in establishing these ARH programmes successfully. Providers' attitude and actions may be a barrier for adolescents in using reproductive health services. A number of studies on human reproduction have explored about the obstacles young people face in obtaining appropriate and timely service relating to contraceptives and other sexual health matters. Some of these obstacles are linked to health facilities and include threatening attitude of health care providers [5]. Their knowledge and perception on ARH become major concern in health care delivery for ARH problems. WHO has also highlighted to perform a situation analysis of adolescent reproductive health in which perception and belief of service providers on adolescent health need should be explored for further health promotion and desirable actions [6].

Therefore, a cross-sectional survey was conducted to inquire the providers' knowledge, perception and the problems encountered in relation to provision of ARH services to impart a valuable input to the future RH programmes aiming at adolescents' health.

MATERIALS AND METHODS

The proposal was approved by the Institutional Review Board of Department of Medical Research (Lower Myanmar) before the study was conducted.

Quantitative survey

Of 20 townships in which ARH corners have been launched, two townships were randomly selected as the study setting by using a computer generated random number, which resulted in Dala and Oak-twin townships in Yangon and Bago Divisions. Quantitative data were collected from all basic Health Staff (BHS) from the study townships by using a pre-tested self-administered questionnaire regarding background characteristics of the BHS, service and training related factors, knowledge on ARH issues including reproductive health physiology, adolescent pregnancy,

contraception, HIV/ AIDS and problems encountered in providing ARH services. A total of 66 BHS participated in the questionnaire survey.

Data cleaning, coding and analysis were done using SPSS 11.5 software. Descriptive statistics were shown as mean/median for continuous variables and percentage for categorical variables. Knowledge items were given a score of "1" to correct answers and "0" for incorrect answers. Then total score was calculated for each ARH issue and categorized into "low" and "high" knowledge by using median score as cut point and described as percentage.

Qualitative survey

Four focus group discussions (FGD), 8 in-depth interviews (IDI) and 4 key informant interviews (KII) were also conducted for qualitative assessment. Two FGD were conducted with midwife (MW) and two were conducted with health assistant (HA) and lady health visitor (LHV). A total of 36 participants were included in four sessions. Eight IDI were done with BHS from the RHC having ARH corner. A total of four KII were carried out with peer youth leaders. Qualitative assessment was focused on their perspectives in giving general ARH knowledge, contraceptive knowledge and HIV/ AIDS knowledge to the adolescents in their community and the problems they have encountered.

After completion of all fieldworks, the interviews were transcribed and organized according to the major themes and sub-themes. Matrix analysis was done after the transcription. Triangulation of research methods was done to validate the findings.

RESULTS

Background characteristics

A total of 66 BHS, 41 (62.1%) BHS from Oak-twin Township and 25 (37.9%) from Dala Township included in the study. More than half were MW (59.1%) and others were HA (13.6%), LHV (12.1%) and nurses (15.2%). Mean age of the BHS was 36.6

years and ranged from 21 to 57 years. Total service ranged from less than one year to 40 years with the median of 7 years. Regarding their educational level, 52% were university graduates and 42% were high school passed. About two-third (67%) got training concerning ARH in which 32% had received more than once.

Knowledge level of Basic Health Staff on adolescent reproductive health issues

Fig. 1 shows the knowledge level of BHS on selected ARH issues. Higher knowledge level was found in relation to HIV/AIDS (84.8%). Only 44% had high level of knowledge regarding general reproductive health.

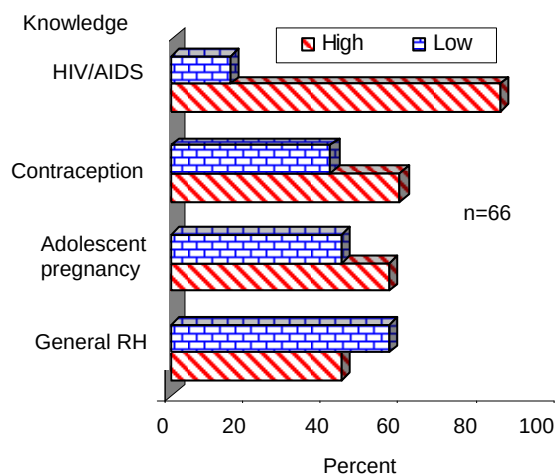
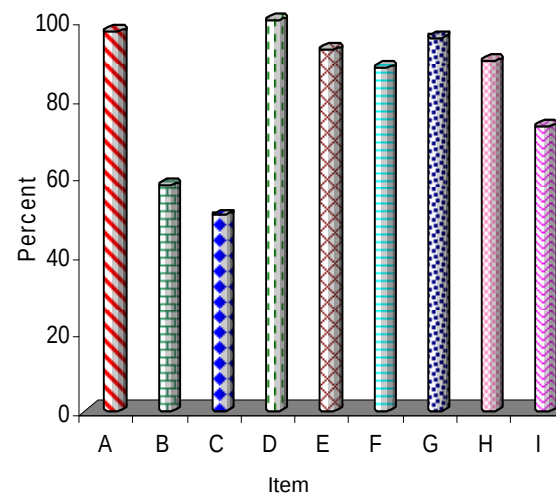


Fig. 1. Knowledge level of BHS on ARH issues

Proportion of BHS who could answer correctly on knowledge items regarding ARH is shown in Fig. 2. All BHS recognized the advantages of condoms while 72% knew the correct timing of emergency oral contraceptives (EOC) administration. However, only 50% of BHS could answer the ovulation period correctly.

Qualitative findings

Most of the participants knew the definition of adolescents with respect to age. Regarding the common ARH problems, more than half could mention the problems in detail. They mentioned early marriage and pregnancy, adolescent child birth, abortion and sexually transmitted infections (STIs)



- A = Adolescent pregnancy risk
- B = Chance of LBW babies
- C = Ovulation period
- D = Advantages of condom
- E = Types of contraceptives
- F = Chance of pregnancy
- G = Chance of HIV infection
- H = HIV and STDs
- I = EOC administration

Fig. 2. Proportion of BHS who could answer correctly on knowledge items

as the most common problems. However, they thought adolescent child birth and abortions were the two most important and common problems within their community. Many could discuss in detail on adolescent puberty changes. Some of them said they had experience about the adolescent pregnancy. More than half could identify the adverse consequences of adolescent pregnancy. Almost all of them said that they had never seen the case of STIs and HIV in the adolescents within their community.

Providers' perspective on giving ARH knowledge

More than half agreed to provide reproductive health information to the adolescents. However, they were also reluctant to inform as it might lead adolescents to become sexually active. A Few stated that they should not tell these reproductive health matters to the adolescents under 15 years of age.

Some of their responses were:

“...I’ll give the information about RH. Otherwise, abortion rate will increase. Telling this knowledge could help to prevent that ...”

(32 years old MW, graduate, 7 years service)

“...I’ve worried since RH training was started for the adolescents. If they know everything, unwanted issues will happen. I think we shouldn’t tell them...”

(55 years old LHV, 10th Std, 30 years service)

“...up to 13 years is quite young. They may be in the 6th or 7th grade. We should give such kind of knowledge starting from 9th or 10th grade...”

(40 years old MW, 10th Std., 18 years service)

Providers’ perspective on giving contraceptive knowledge

Some providers agreed to impart the contraceptive knowledge to all adolescents. However, some stated that they would like to give this knowledge only to the married adolescents and after 16 years of age. Most of them were hesitant to tell this information to unmarried adolescents.

“...We do educate them about condom because of HIV/AIDS problem. Using condom could ensure for prevention but other methods could not guarantee....”

(51 years old MW, 10th Std, 23 years service)

“...If unmarried girl asked about contraception, we should inquire whether she’s a bad girl (sex worker) or not. We should inform the parents according to the condition.”

(46 years old MW, graduate, 19 years service)

Problems encountered and strategies to overcome in providing ARH services

Most providers (71%) had experience of providing ARH services especially giving reproductive health education and

counselling in the villages under their jurisdiction. They have been carrying out the activity with the help of youths and community support group (CSG). It was mostly conducted as group education in appropriate places. About one-third (32%) encountered difficulty in recruiting the adolescents. Majority mentioned that giving reproductive health (RH) education depends on the background education level of the adolescents especially in rural area. In addition, most of the adolescents were very shy and reluctant to listen to RH matters. Unwillingness of parents, teachers and village leaders to equip the adolescents with RH knowledge was seen in some places. Most of them thought that it was not necessary for the adolescents to have RH knowledge.

Consequently, providers used the strategy of giving RH information with other health topics about infectious diseases (e.g malaria) in appropriate places such as “Saylate-khone” (Cheroot-production work place) where many adolescents work for their living. Some providers mentioned that they could not spare much time for ARH services as they have many duties to perform for various diseases.

“...Now, we don’t give separate RH education. As they are very shy...we’ve to start with other health issues like malaria. After that we moved to the RH topic....”

(55 years old HA, 10th Std, 33 years service)

Youth leaders’ perspective on ARH services

Youth leaders also revealed their perception about ARH services. They mentioned that RH information should be provided to all adolescents. All youth leaders stated that most adolescents seem reluctant to use ARH corner as it is situated in a RHC where many adults come and seek health care although most providers are willing to provide the service. In addition, they also thought that most adolescents were afraid of breach of confidentiality as other adults could report to their parents. According to their experience, most adolescents come to

the corner on holidays to read the books but they were reluctant to discuss RH matters with the health care providers.

“...Most adolescents don’t go to the health center as they are afraid that their parents will know about that...”

(20 years old, Female, 10th Std)

DISCUSSION

Our study highlights the knowledge and perception of BHS on ARH as well as the problems encountered in providing these services. Majority had high level of knowledge regarding HIV/AIDS. Knowledge on reproductive physiology, adolescent pregnancy and contraception was found to be quite low. Specifically, half of them could not answer the correct time of ovulation in relation to menstrual cycle and about one-third did not know the correct timing of EOC administration. Regarding perception on providing ARH services, most providers were willing to impart RH information to older and married adolescents. Some were reluctant to provide younger adolescents.

Most providers had low level of knowledge on some RH issues. It was quite alarming that only half of the respondents knew the correct ovulation period. Likewise, most health care providers in a study conducted in Swaziland mentioned that they were not adequately equipped with knowledge and skills to provide comprehensive ARH services [7].

Our study identified that most providers were hesitant to tell contraceptive information and service to unmarried adolescents. Moreover, some providers wanted to give contraceptive knowledge only to the married adolescents and above 16 years of age. A study conducted in Swaziland on providers’ perception of adolescent sexual and reproductive health care also found out that some providers did not provide contraceptive service as adolescents should not indulge in sex [7]. Some providers from

our study revealed their unfavourable attitude on providing RH services to unmarried adolescents. This finding was supported by a previous study in which adolescents pointed out the providers’ attitude as the barrier for adolescents in using RH services in Sri Lanka [8].

According to our findings, most adolescents were reluctant to discuss RH matters with health care providers. In Swaziland, although most adolescents favoured information from the health care providers, only few had received information from them [9]. In contrast, adolescents stated health personnel as their preferred source of information about RH matters in a study in Sri Lanka [8].

Adolescents’ fear of breach of confidentiality was one reason mentioned by the peer youth leader. This finding was supported by a study in which most adolescents wanted to use health care without their parents’ knowing [10]. In addition, most adolescents were reluctant to use ARH corner as it is situated in RHC. Likewise, adolescents from Iceland mentioned that location for RH services should be at a separate clinic instead of within community health center or hospital. However, about one-third thought that it should be located within community health center [11]. A study done in Sri Lanka found that use of existing clinics to provide ARH services was accepted by majority of the adolescents [8]. Unwillingness of parents, teachers and village leaders to equip the adolescents with RH knowledge was one problem indicated by the providers. Similarly, negative attitude of parents, teachers and society was one of the barriers mentioned by adolescents from Sri Lanka [8].

In conclusion, most providers were willing to provide RH information to the adolescents though many of them did not want to inform younger and unmarried adolescents. Majority had high level of knowledge regarding HIV/AIDS. In contrast, about half of them had low level of knowledge on reproductive physiology.

Recommendation

1. Refresher training should be provided as more than 50% of BHS had low level of knowledge in some ARH issues.
2. Providers should be encouraged to impart RH information to all adolescents regardless of the age and marital status.
3. Providers' attitude towards unmarried adolescents should be corrected.
4. ARH corner should be arranged in a separate place from RHC to become adolescent-friendly services.

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Learning experiences of student nurses in clinical practice

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This study aimed to explore the learning experiences of the student nurses during their periods of the clinical practice. A survey method was employed with a questionnaire administered to all the final year student nurses of the two nursing universities in Myanmar. The design of the questionnaire was developed through a review and inclusion of key issues from the literature and interviews of ten experienced lecturers and tutors. There were 272 final year student nurses in both universities. All respondents answered that their theoretical training helped their routine clinical practice. The majority of the students (65.8%) felt that they did not have enough time for clinical practice. Some 75.4% experienced with many students in one ward and 57.3% did not have opportunities to talk and discuss with the supervisor. About 65% of the students also experienced the difference between theoretical learning and clinical practice. Most of the students (75.4%) felt that they did not have opportunities to try out each clinical skills, but 57.7% had opportunities to ask questions when they did not understand. About 96% of the student nurses had the opportunities to share the experiences and skills gained from the clinical teaching among the group members. In addition, the student nurses emphasized some influencing factors for learning in clinical practice. Clinical teachers as well as academic staff may reflect on these findings. They could plan to increase integration between theory and practice, and promote and reinforce both formal and informal support and supervision to the students to improve the current skills teaching of nursing practice in Myanmar.

INTRODUCTION

Nursing education is a planned educational programme, which provides a broad and sound foundation for the effective practice of nursing. Education of nurses is fundamental, not only for the public safety but also for the nursing profession to be respected. The goal of nursing educational programme is to prepare graduates for the professional practice of nursing, with nursing care skills that are applicable in any setting. The issues that nursing is facing today and the dynamic nature of nursing practice make it imperative for the nursing education to develop a clinical based experience for baccalaureate students, who are responsive to the social context of nursing. Nursing is a practice-based

profession and therefore, the acquisition of (fundamental) clinical nursing skills is essential [1]. Skill acquisition is an integral part of the development of any practitioner and the student nurses need opportunities to master the skills. In the nursing education or training for the nurses, it is important to emphasize on nursing skills acquisition and attitude development [2]. Clinical teaching provides the opportunities for the students to learn effectively in the application of theories to practices. It also provides experience with real clients and real problems, which help the learners' ability to use the acquired knowledge in practice. In order to produce graduate nurses with high quality, it is needed to have adequate clinical facilities including the provision of professional learning environment which is

conducive for learning. However, in Myanmar although the emerging problem of newly appointed nurses shows that lack of clinical skills has been encountered, in-depth studies and research still need to be conducted into this, to pave the way for improvements in the skills teaching of nursing practices [3].

This study aimed to explore the learning experiences of the student nurses during their periods of the clinical practice. The results from this study could be utilized to improve the current skills teaching of the nursing practice in Myanmar.

MATERIALS AND METHODS

A cross-sectional study was conducted by employing self-administered questionnaire to all the final year student nurses of the two nursing universities in Myanmar. The design of the questionnaire was developed through a review and inclusion of key issues from the literature and interviews of ten experienced lecturers and tutors. The questionnaire was designed to include both closed and open-ended questions in order to gather more detailed information. There were 272 final year student nurses in both universities, but 260 students (95.6%) responded the questionnaire. Each question was coded in number and then entered into the computer by using Epi data software version 3.0. Data were edited by logical and consequence checking. Then it was analyzed by using SPSS version 11.5.

The four open-ended questions were included in the questionnaires. The responses were analyzed by content analysis. The findings were summarized in terms of three enhancing and three inhibiting categories, which were presented using the six categories as subheadings:

(A) Enhancing: supervision in clinical practice, opportunity to practice, conducive learning environment;

(B) Inhibiting: insufficient supervision in clinical practice, lack of opportunity

to practice, lack of conducive learning environment.

The categories consisted of 13 enhancing and 10 inhibiting factors in clinical practice. The contents of the factors were verbally described with some verbatim examples.

To get permission to conduct this research with the final year student nurses from two nursing universities, the researchers discussed with the administrative personnel from the Department of Medical Sciences and the universities of nursing. In addition, all participants (student nurses) were informed about the nature of the research, objectives of the study and the content of the questionnaire. All potential participants were informed that participation is voluntary and they have the autonomy to terminate their participation whenever they wish to. They were guaranteed confidentiality and anonymity for questionnaire data.

RESULTS

The majority of the students (65.4%) were 20 years old. Female composition was 95.7% and only 12 out of 260 (4.3%) were male students.

Learning theoretical lessons

All respondents answered that their theoretical training helps the routine clinical practice. About 75% of the students answered that more than 50% of theoretical training could be applied in clinical practice.

Assignment to clinical practice

More than half of the students (65.8%) answered that they had too little time for clinical practice. About one third (27.3%) said that the duration for clinical practice was just right. Three quarters of students (75.4%) answered that too many students were assigned in one ward. More than half of the students (60%) answered that only 5-10 students should be assigned in one ward. About one third of the students (37.7%) said that less than 5 students should be assigned in one ward.

The supervisor was the ward sister or staff nurse who supervised the group of student nurses in clinical setting. One third of the students (37.3%) had an opportunity to discuss with the supervisor on a one-to-one basis sometimes and 108 students (41.5%) rarely got such an opportunity. Although 106 students (40.8%) sometimes received the opportunities for small group discussions with the supervisor, 89 students (34.2%) got rarely.

Of the 260 respondents, 47 students (18.1%) answered that they never received them. Of the 260 respondents, 111 students (42.7%) got sometimes and 92 students (35.4%) rarely got opportunities for large group discussions with the supervisor. Thirty-eight students (14.6%) answered that they never experienced them. The result indicated that learning from discussion with the supervisor was not a regular event for them.

Practicing clinical skills

The majority of the students (64.6%) answered that they often experienced the difference between learning theory in the university and its practice in the clinical wards. For example, more than half of the students (54.6%) answered that they experienced with the use of improper technique because of insufficient instruments, equipment and facilities.

The following table shows student nurses' responses on factors which hindered clinical learning.

Table 1. Factors which hindered clinical learning

	Frequency	Percent
Feeling unprepared for clinical practice	78	30.0
Feeling incompetent in clinical skills	128	49.2
Fear of doing harm to patients	164	63.1
Having sense of not belonging to the nursing team	11	4.2
Not having enough facilities to practice	176	67.7
Others including numerous students in one ward, theory-practice gap	95	36.5

Note: Total does not add up to 100 due to multiple responses.

Although about 63% of the students responded that they sometimes got enough opportunities to try out clinical skills and about one quarter of the students (23.8%) often received these opportunities.

More than half of the students (56.9%) responded that they often received opportunities to ask questions when they did not understand in the clinical practice and 100 students (38.5%) sometimes got these opportunities.

Ninety-eight students (37.7%) often received opportunities to develop new knowledge, concepts and ideas during clinical practice and 89 students (34.2%) answered that they and 89 students (34.2%) answered that they sometimes got these opportunities.

Regarding the question on "Do you have opportunities to share the experiences and skills gained from the clinical teaching among the group members ?", 94 students (36.2%) always received, 96 students (36.9%) often received and 59 students (22.7%) sometimes received these opportunities (Fig.1).

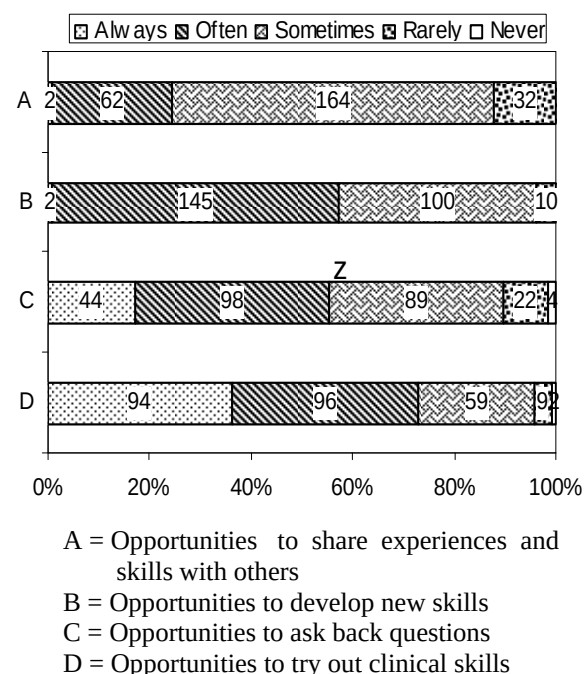


Fig. 1. Different opportunities to practice clinical skills

The role of supervisor in clinical practice

About one third of the students (35.4%) said they often had good relationship with the

supervisor, while 45% sometimes had such relationship. While 115 students (44.4%) answered that they could learn attitudes and values of a nurse from the supervisor sometimes, 59 students (22.8%) could rarely learn.

One third of the students (31.9%) answered that the supervisor could often explain well but about half of the students (46.9%) said the supervisor could sometimes explain when they asked the questions. Although 44.2% felt that the supervisor sometimes demonstrated procedures/care practices effectively and 73 students (28.1%) said the supervisor rarely did.

About one third of the students (31.5%) sometimes received immediate and positive feedback from the supervisor and 111 students (42.7%) rarely received the feedback. Some students (42.3%) said the supervisor often had ample background knowledge but 34.2% answered sometimes.

Regarding the up-to-date knowledge of the supervisor, 42.4% felt that the supervisor often had up-to-date knowledge but about one third of students (34.2%) answered that the supervisor sometimes had up-to-date knowledge.

Concerning the clinical competence of the supervisor, about one third of the students (31.5%) answered the supervisor always had clinical competence and about half of the students (46.5%) said that the supervisor often had clinical competence. Although 40.4% answered that the supervisor sometimes assisted the learners in relating underlying theory to nursing practice, 35% said the supervisor rarely did.

Students' perspective on supervisor in clinical practice

The followings are enhancing and inhibiting factors affecting for learning in clinical practice (Fig. 2).

Enhancing factors

Supervision in clinical practice

- Supervision and monitoring of practice by the supervisor and the ward staff

- Good student-supervisor relationship
- Supervisor's interest in clinical teaching
- Teaching of clinical tutor in the ward
- Constructive feedback from the supervisor

Opportunity to practice

- Allowing to do nursing tasks independently
- Frequent bedside teaching
- Giving enough time for the clinical practice
- Assigning appropriate number of students in one ward
- Giving opportunity to discuss and practice within small group

Conducive learning environment

- Providing adequate instruments and equipments in the ward, sufficient learning resources
- Getting chance of group discussions
- Creating warmth and good relationship from the supervisor and the ward staff

Some examples of students' answers were as follows;

One student expressed:

"I have inserted many intravenous lines and I have also performed nasogastric tube insertion and catheterization and they really helped me to gain confidence in the clinical practice".

One student revealed:

"If the supervisor and the ward staff treat the nursing students fairly and are considerate, it can help a great deal in the clinical practice".

One student said:

"If the ward sister and the ward staff can give us a warm welcome, and also the clinical tutors from the university will join in the bedside teaching and group discussions, our clinical learning will be more interesting and enjoyable".

One student expressed:

"We can learn nursing skills by doing so that we are working in the ward in order to learn. Clinical learning would be more

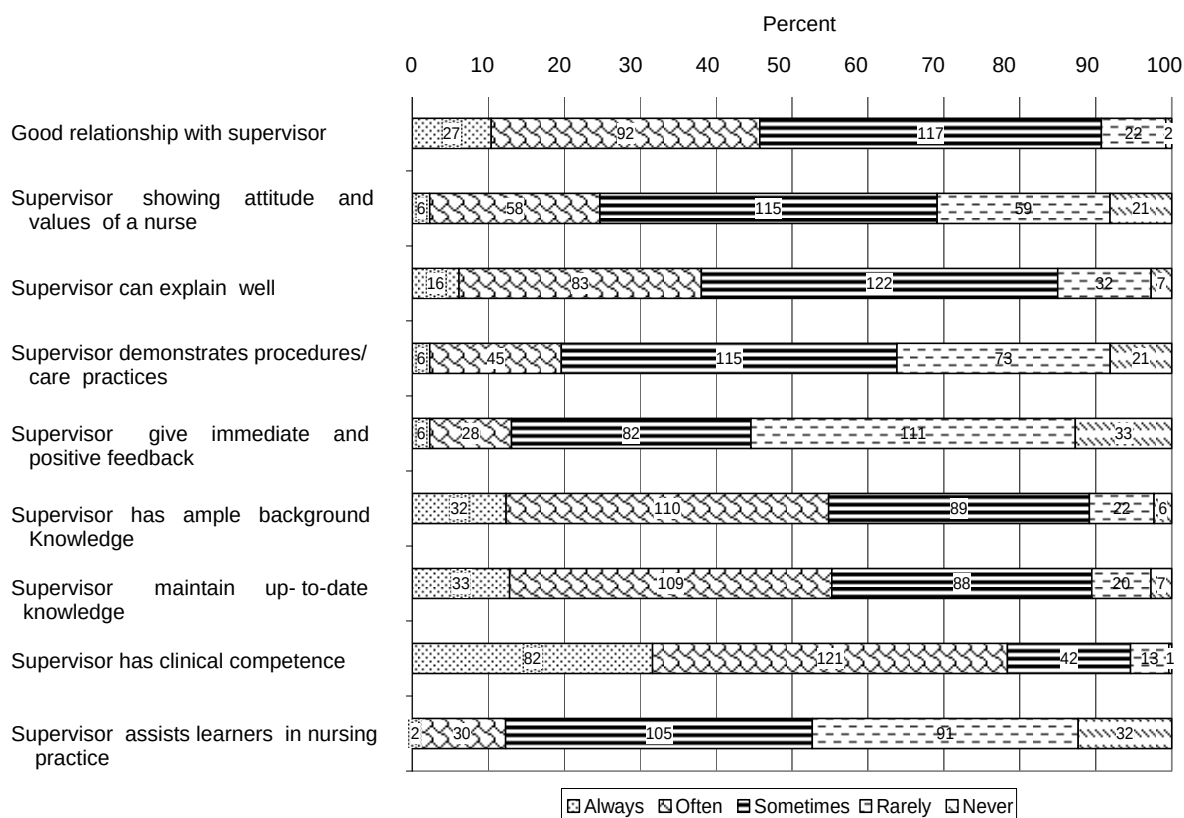


Fig. 2. Students' perspective on supervisor in clinical practice

effective and enjoyable if we got proper supervision while performing the nursing procedures”.

Inhibiting factors

Insufficient supervision

- Insufficient supervision and monitoring of practice by the supervisor
- Poor student-supervisor relationship
- The supervisor's lack of interest in clinical teaching
- Lack of constructive feedback from the supervisor

Lack of opportunity to practice

- Lack of opportunities to practice allowing only to watch, not to participate, in the care of patients
- Inadequate practicing time
- Assigning many students in one ward

Lack of conducive learning environment

- Providing inadequate instruments and equipment in the ward; insufficient learning resources

- Treating differently between diploma and bachelor students
- Shouting and blaming in the presence of the patients when students made mistakes

One student said:

“Because of the shortage of the nursing manpower, sometimes there are only two or three nurses in the morning shift. They are always busy with the routine activities in the ward, so that teaching and supervising the students in the ward is ineffective”.

One revealed:

“The supervisor is shouting and irritating without any reason. When questions are asked, she thinks it is disturbing, it can also make the clinical learning difficult. I think that the good relationship between the supervisor and the students is a major influence in effective clinical learning in the ward”

One student expressed:

“We are not able to practice the procedures taught at the university in the ward because of shortage of equipment”.

DISCUSSION

The key findings of this study pointed out some problems which affect the students' clinical learning such as: theory-practice gap, insufficient duration of time for clinical practice, many students in one ward, lack of opportunities to talk and discuss with the supervisor, not only on the one-to-one basis but also within small group and large group, lack of opportunities to try out each clinical skills, poor student-supervisor relationship and the supervisor's clinical teaching. The student nurses emphasized supervision in clinical practice, opportunity to practice and conducive learning environment as enhancing categories which consist of 13 enhancing factors in clinical practice. The student nurses also emphasized insufficient supervision in clinical practice, lack of opportunity to practice and lack of conducive learning environment as inhibiting categories which consist of 10 inhibiting factors in clinical practice as shown in the results section.

All the respondents answered that their theoretical training helped their routine clinical practice. About 75% answered that more than 50% of the theoretical training could be applied in the clinical practice. The majority of the students (64.6%) answered that they often experienced the difference between learning theory in the university and its practice in the clinical wards. In this study, most of the students also expressed the view that shortage of instruments and equipment in the clinical areas was a major reason why it is difficult to adjust theory taught in the university to practical work during the clinical learning. It could be said that the environment of the classroom, where most of the theoretical input is taught, can never truly resemble the real situation and that a full comprehension of the nursing principles does not ensure their application to practice [4].

It would seem that the theory-practice gap is a multifaceted problem. It seems that

striving for deeper understanding of the roles nurses play in a changing educational environment is a constant challenge [5]. It is necessary to avoid traditional strategies and the supervisor needs to carefully evaluate new initiatives [5]. Integration of theory and practice in the nurse education programme is very important, so careful thought is required for the curriculum design [5].

Many of the basic prerequisites for clinical practice, learning by doing, experiencing the patient relationship, were not fulfilled for these student nurses. The student nurses in this study revealed negative experience with the supervisor. Supervision and monitoring of practice by the supervisor and ward staff, good student-supervisor relationship, getting constructive feedback, and adequate instruments and equipment in the ward, sufficient learning resources and conducive learning environment, were emphasized as inhibiting factors. These factors highlight the importance that students attached to the willingness of the supervisors to engage in a teaching relationship. The supervisors and clinical staff are of special importance in the final part of the student nurses' education to facilitate transition to the professional role [6].

Around 55% of student nurses in this study expressed that they rarely or never received immediate and positive feedback from the supervisor (Fig. 2). Students want feedback about their performance to gauge progress concerning knowledge, competence, and faculty expectations [7], and rate giving feedback as an essential quality of an effective clinical teacher [8]. There is evidence to suggest that feedback is an essential ingredient for effective learning. Research in skills acquisition has shown that students learn best if feedback is incorporated [9]. Therefore, incorporating feedback on students' performance is an important principle when designing clinical teaching programme.

The clinical learning environment encompasses all that surround the student nurse, including the clinical settings, the equip-

ment, the patients, the clinical staff, the nurse mentor, and the nurse teacher [10]. A good clinical learning environment could be established through good co-operation between the school or university and clinical staff.

Conclusion and recommendations

This study provided what the student nurses experienced during their clinical education. The student nurses laid emphasis on those areas that nurse educationalists must refer to, in order to improve the quality of clinical education. The findings of this study give color to the nature of learning experiences in clinical practice from final year student nurses' point of view.

In Myanmar, while the number of students increased in tandem with the Human Resources Development, the total appointed teaching staff and clinical staff remained static. On the other hand, hospital staff are over burdened with their clinical duties. They found difficult to cope with the simultaneous teaching and the clinical responsibilities to make up for any shortfalls in the skills learning of the student nurses. Thus, teaching and supervising the students in the ward become very weak. The standard of nursing care practice, good atmosphere and conducive for clinical learning situations, all depend on the ward sister and clinical staff. They can make smoothness of the clinical teaching for academic staff. Therefore, clinical staff's contribution to nursing education especially for clinical teaching is crucial and very important.

Clinical teachers as well as academic staff could reflect on these findings to improve the quality of nursing education particularly for the following recommendations.

1. Integration between theory and practice should be increased and students' adaptation processes should be facilitated.
2. An effective means of reinforcement like supervision should be part of the curriculum in any form of nursing education.

3. All the learning activities intended for skills development should allow opportunities for repeated practice.
4. The ward should be provided with adequate facilities and the equipment required for performing nursing skills.
5. Research that focuses on nursing manpower and its utilization in the hospital wards should be conducted, and the research findings should be submitted to the decision-makers for appropriate action.

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Plasma malondialdehyde, vitamin C and vitamin E levels of normal pregnant and preeclamptic pregnant women in West Yangon General Hospital

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This study was conducted to evaluate the plasma levels of vitamin C, vitamin E, and malondialdehyde (MDA) in 48 normal pregnant women and 35 preeclamptic Myanmar pregnant women between the ages of 18-40 years and at the gestational ages of ≥ 32 weeks. Plasma levels of vitamin C, vitamin E and MDA were measured by spectrophotometric methods. In normal pregnancy, plasma vitamin C level was 1.25 ± 0.13 mg/dl, plasma vitamin E level was 1.88 ± 0.47 mg/dl and plasma MDA level was 2.02 ± 0.25 $\mu\text{mol/l}$. In preeclampsia, plasma vitamin C level was 0.73 ± 0.11 mg/dl, plasma vitamin E level was 1.33 ± 0.39 mg/dl and plasma MDA level was 3.20 ± 0.30 $\mu\text{mol/l}$. Plasma levels of antioxidant vitamins C and E were significantly lower in preeclamptic pregnancy than in normal pregnancy. Plasma MDA level was significantly higher in preeclamptic pregnancy than that of normal pregnant women. There were significant negative correlations between plasma MDA level and plasma levels of vitamin C and vitamin E in normotensive as well as in preeclamptic pregnant women. A significant correlation was also detected between diastolic blood pressure and plasma levels of vitamin C, vitamin E and MDA in normal pregnancy and in preeclampsia. In conclusion, plasma MDA level was increased and antioxidant vitamins C and E were decreased in preeclampsia.

INTRODUCTION

Pregnancy is a stressful condition in which many physiological and metabolic functions are altered to a considerable extent. Preeclampsia is one of the most serious complications of pregnancy and despite extensive research its etiology and pathogenesis remain obscure.

Normal pregnancy itself is an oxidative event as it has been demonstrated that circulating levels of lipid peroxides increase significantly during the gestation when compared to that of non-pregnancy [1]. It indicates that peroxidation reactions are enhanced during pregnancy. The major source of oxidative stress during gestation appears to occur in the placenta. Generation of free radicals increases during pregnancy

and placental mitochondria are the major sources of reactive oxygen species. Reactive oxygen species attack the phospholipids of cell membranes and react with polyunsaturated fatty acids, occurring lipid peroxidation, which results in primary lipid peroxidation products such as lipid hydroperoxides and secondary lipid peroxidation products such as MDA. Lipid hydroperoxides bind to lipoproteins and are carried to distant sites where the hydroperoxides can propagate lipid peroxidation.

The destructive process of lipid peroxidation may contribute to the development of vascular endothelial cell dysfunction. The elevated plasma concentration of MDA may precede the development of preeclampsia. Lipid peroxidation products are toxic to cells and membranes, but they are normally

controlled by the antioxidant scavenging system. Uncontrolled peroxidation may cause disruption of membrane lipids and other cell components, and then lipid peroxides are involved in endothelial cell injury, vasoconstriction, and imbalance between thromboxane and prostacyclin in preeclampsia.

In addition to the enzymic mechanism of the free radical removal, essential nutrients that can scavenge free radicals, such as vitamin C and E, constitute a strong line of defense in retarding free radical induced cellular damage. Antioxidant systems serve to protect them against the harmful oxidative reactions. However, chronic excessive generation of free radicals will deplete antioxidant vitamins.

Several studies have reported that there is an imbalance between lipid peroxides and antioxidants in preeclampsia and suggested that oxidative stress may contribute to the damage of endothelium which plays a role in the pathophysiologic mechanism of the disease [2-5].

The present study is carried out to determine plasma levels of MDA, vitamin C and vitamin E in normal pregnant and pre-eclamptic pregnant women. The outcome of the research may be useful in the determination of the need to give antioxidant vitamins in normal pregnancy as well as in preeclampsia.

Objectives

- To determine the plasma ascorbic acid level of normal pregnant and pre-eclamptic pregnant women in West Yangon General Hospital (WYGH)
- To determine the plasma tocopherol level of normal pregnant and pre-eclamptic pregnant women in WYGH
- To evaluate the plasma MDA level of normal pregnant and pre-eclamptic pregnant women in WYGH
- To compare the above biochemical parameters in preeclampsia with normal pregnancy

MATERIALS AND METHODS

A hospital-based study was performed on 48 normal pregnant women and 35 pre-eclamptic pregnant women presenting at WYGH antenatal clinic. They were interviewed and thorough clinical history was taken after their informed consents had been obtained. Their blood and urine samples were collected before starting any anti-hypertensive medication. All biochemical parameters; plasma vitamin C, vitamin E, and MDA levels were determined at the Research Laboratory of Biochemistry Department, University of Medicine I, Yangon, on the same day of sample collection. All samples were collected with code numbers and analyzed batch by batch in duplicate. Plasma vitamin C level was determined by simple colorimetric method for ascorbic acid [6]. Plasma vitamin E level was determined by spectrophotometric method [7]. Plasma MDA level was determined by spectrophotometric method using thiobarbituric acid [8].

Operational definition of preeclampsia is as followed: hypertension of at least 140/90 mmHg recorded on two separate occasions at least 4 hours apart and in the presence of at least 300 mg protein in a 24-hour collection of urine (1+ proteinuria), arising de novo after the 20th week of gestation in a previously normotensive woman and resolving completely by the 6th postpartum week [9].

Inclusion criteria for the present study were age between 18-40 years and 28-40 weeks gestational period at blood and urine sampling. Exclusion criteria were women in labour, multiple pregnancy, smoker, those taking antioxidant vitamins, and any concurrent medical diseases.

Data analysis was done by using the Statistical Package for Social Sciences (SPSS) software version 13. Standard statistical methods were applied for the calculation of mean, standard deviation and standard error. Student's "t" test (unpaired) was applied to calculate the significance of

difference between the means on 95% confidence interval of each parameter. Evaluation was done at the probability level of less than 0.05. Pearson's correlation coefficient (r) was calculated to assess the relationship between the variables.

RESULTS

Basic parameters

Both normal pregnant women and preeclamptic pregnant women had gravida 1 to 3. Systolic and diastolic blood pressures were significantly high in preeclamptic pregnant women (Table 1).

Table 1. Clinical parameters of normal pregnant and preeclamptic pregnant women

	Normal pregnant women ^a (n = 48)	Preeclamptic pregnant women ^a (n = 35)	Level of significance ^b (p)
Age (years)	28.02±6.05	28.31±5.28	0.819
Gestational age at blood sampling (weeks)	35.58±1.76	35.57±1.48	0.974
Systolic blood pressure (mmHg)	117.71±4.25	144.57±6.11	<0.001*
Diastolic blood pressure (mmHg)	77.71±4.25	94.29±5.02	<0.001*

a=The data shown are as mean ± standard deviation values.

b=The p values refer to the difference from the normal pregnant group.

*=denotes statistically significant according to Student's t-test.

Plasma vitamin C, vitamin E, and MDA levels

Plasma vitamin C, vitamin E, and MDA levels of normal pregnant and preeclamptic pregnant women are shown in Table 2. Mean plasma levels of vitamin C and vitamin E were significantly lower in preeclamptic pregnant women as compared to normal pregnant women. Mean plasma MDA level was significantly higher in preeclamptic pregnant women than that in normal pregnant women.

Correlation between plasma MDA level and plasma levels of vitamin C and vitamin E

There was a significant negative correlation

Table 2. Plasma vitamin C, vitamin E and MDA levels of normal pregnant and preeclamptic pregnant women

	Normal pregnant women ^a (n = 48)	Preeclamptic pregnant women ^a (n = 35)	Level of significance ^b (p)
Plasma vitamin C level (mg/dl)	1.25±0.13	0.73±0.11	0.001*
Plasma vitamin E level (mg/dl)	1.88±0.47	1.33±0.39	0.001*
Plasma MDA level (μmol/l)	2.02±0.25	3.20±0.30	0.001*

a=The data shown are as mean ± standard deviation values.

b=P values refer to the difference from the normal pregnant group.

*=Statistically significant according to student's t test

between plasma MDA level and plasma vitamin C level in normotensive pregnant women ($r=-0.921$, $p<0.001$) and in preeclamptic patients ($r=-0.957$, $p<0.001$). There was a significant negative correlation between plasma MDA level and plasma vitamin E level in normotensive pregnant women ($r=-0.845$, $p<0.001$) and in preeclamptic patients ($r=-0.875$, $p<0.001$).

Correlation between plasma level of MDA, vitamin C and vitamin E with blood pressure

Plasma MDA level was found to be significantly and positively correlated with diastolic blood pressure in normal pregnant women as well as in preeclamptic pregnant women ($p<0.01$ and $p<0.05$, respectively).

Plasma vitamin C level was found to be significantly and negatively correlated with diastolic blood pressure in normal pregnant women as well as in preeclamptic pregnant women ($p<0.01$ and $p<0.05$, respectively).

Plasma vitamin E level was found to be significantly and negatively correlated with diastolic blood pressure in normal pregnant women as well as in preeclamptic pregnant women ($p<0.01$ and $p<0.05$, respectively). Plasma vitamin E level was also significantly and negatively correlated with systolic blood pressure in normal pregnant women ($p<0.05$).

DISCUSSION

Free radicals by their unstable and transient nature are difficult to measure directly. Their tendency to cause lipid peroxidation has been used as an indirect measure. Marker of lipid peroxidation (MDA) was found to be increased in preeclamptic pregnancy. A number of reports also indicated that plasma level of MDA, lipid peroxidation product, was elevated in women with preeclampsia relative to normal pregnancy, and it has been suggested that lipid peroxidation may play a role in the etiology of the disease [10-13].

Plasma vitamin C level of preeclamptic pregnant women was significantly lower than that of normal pregnant women and it might be due to increased utilization of vitamin C to scavenge free radicals and also to regenerate other antioxidants in preeclampsia.

Plasma vitamin E level was also significantly lower in women with preeclampsia than that in normal pregnancy. Decreased plasma vitamin E level in preeclampsia may be due to increased consumption of the vitamin during lipid peroxidation. Another possibility is decreased absorption of vitamin E from the gut as a result of vasoconstriction in preeclampsia.

Antioxidant nutrients counteract the free radical disturbances and thereby protect cell membranes against free radical mediated lipid peroxidation. As a water-soluble antioxidant, ascorbic acid traps most of the free radicals present in the aqueous phase of the plasma and functions as a first-line defense mechanism against free oxygen radicals. When the capacity of ascorbic acid is exceeded, free radicals can then diffuse to the cell membranes. Vitamin E is highly lipid-soluble and is distributed in the lipid layer of the membrane and acts as a free radical scavenger in preventing lipid peroxidation in the membrane of cells, mitochondria and cell organelles where lipid peroxidation occurs. Preeclampsia is thus

associated with increased utilization of antioxidant vitamins.

The increase in oxidative stress was further supported by the observation of a negative correlation between lipid peroxidation product (MDA) with antioxidants (vitamin E and vitamin C).

An increase in diastolic blood pressure correlates positively with plasma MDA level and also correlates negatively with plasma vitamin C and vitamin E levels indicating that the severity of hypertension in preeclampsia was correlated with the extent of lipid peroxidation.

The findings of the present study are in agreement with the study including 30 preeclamptic pregnant women and 30 normotensive pregnant women in India [14]. Mean plasma vitamin C level was 1.06 ± 0.41 mg/dl in normal pregnancy and 0.81 ± 0.1 mg/dl in preeclampsia. Mean plasma vitamin E level was 1.12 ± 0.05 mg/dl in normal pregnancy and 0.5 ± 0.04 mg/dl in preeclampsia. Both plasma vitamin C and vitamin E levels were significantly decreased in preeclampsia as compared to controls ($p < 0.05$).

In another study, assessment of plasma vitamin C and MDA levels in 20 normal pregnant and 38 preeclamptic pregnant women was carried out [4]. Plasma vitamin C level was significantly lower and plasma MDA level was significantly higher in preeclamptic pregnancy compared with normal pregnancy. The present study also agreed well with that result.

The results of the present study are also in agreement with a study in Japanese women [15]. Serum MDA and vitamin E levels were measured in 18 preeclamptic and 25 normotensive pregnant women in Ondokuz Mayıs University Hospital. The investigators concluded that there was an imbalance between lipid peroxidation and serum vitamin E level in preeclampsia and that increased lipid peroxidation was well correlated with the increase in systolic and diastolic blood pressure measurements.

The results of the present study suggested that vascular endothelial cell dysfunction in preeclampsia may be caused by uncontrolled lipid peroxidation which overwhelms the protective mechanisms of the antioxidants. In other words, the decrease in antioxidant vitamin levels observed in the present study indirectly supports the concept that free radical mediated lipid peroxidation and related antioxidant consumption may be involved in the pathophysiologic mechanisms of preeclampsia. The concept of increased utilization of vitamin C and vitamin E in preeclamptic pregnant women raises the possibility of a potential protective role for antioxidant vitamins for preeclampsia.

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Development of cytogenetic method for identification of *Anopheles culicifacies* species complex and its applications at malaria endemic areas of Myanmar

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Cytogenetic method for detailed identification of *An. culicifacies* was developed and tested in the field and laboratory. The objective of the study was to develop polytene chromosome cytogenetic method for *An. culicifacies* and to identify its sibling species complex by ovarian nurse cell polytene chromosome technique in malaria endemic areas of Myanmar. The study was conducted in two endemic areas, Paukkaung Township, Bago Division and Madaya Township, Mandalay Division. Adult mosquitoes were collected by animal baited big net trap and WHO recommended sucking tube in the field. Blood fed *An. culicifacies* were kept for 12 hours, 24 hours and 36 hours and ovaries were dissected and fixed in 10% methyl alcohol and ethyl alcohol fixatives in the field. In main laboratory, they were kept at 4°C in refrigerator. Ovarian nurse cell chromosome bands were examined by compound microscope under 400x magnifications. Blood fed *An. culicifacies* accounting 613 specimens from Bago Division and 35 specimens from Mandalay Division were examined and the result revealed that both methyl alcohol and ethyl alcohol were found to be suitable fixatives and preservatives for prolong period. Up to 70% to 80% of clear and accurate polytene chromosome bands were detected from the 36 hours aged ovary group. Furthermore, sibling species of *An. culicifacies* were studied in detail by using this cytogenetic method and found to be type "B" of *An. culicifacies* complex. It is assumed that in Myanmar type B species are abundant in Bago and Mandalay Divisions. However, further study is needed to find out the presence of other type of species complexes in endemic areas. Although *An. culicifacies* is not a primary vector in Myanmar, it has been abundantly present in association with primary vectors such as *An. dirus* and *An. minimus* in endemic areas. Better understanding of species complex distribution and the role in malaria transmission will be beneficial in prevention and control of malaria.

INTRODUCTION

In Myanmar, 37 *Anopheles* mosquito species were recorded and *An. dirus* and *An. minimus* are major vectors of malaria. Secondary vectors, namely *An. culicifacies*, *An. annularis*, *An. maculatus* are abundant in central and lower Myanmar. Khin Maung Kyi [1] stated that *An. culicifacies* is not a vector of malaria in hyperendemic foothill areas of Myanmar. *An. culicifacies* was the earliest to be identified amongst the Myzomyia series of *Anopheles* (Cellia)

in Thailand [2]. *An. culicifacies* is regarded as the malaria vector in Sri Lanka [3, 4] and cytotaxonomical identification of *An. culicifacies* from Southern Sri Lanka identified it as species B [5]. In South East Asia, there are 4 sibling species of *An. culicifacies* comprising species A and B [5], species C [6], and species D [7, 8]. Species A, C and D are main vectors of malaria and B was a poor vector of malaria in India and Pakistan [9, 10]. Vector potential of these species varies depending on the region. Sibling species of *An. dirus*

were identified by salivary gland polytene chromosome method at the Medical Entomology Research Division (MERD), Department of Medical Research (Lower Myanmar) (DMR-LM). Morphological identification techniques are unable to distinguish among sibling species. Each sibling species may have its own distribution, biology, and may vary in response to insecticides and capacity to transmit malaria. It is important to develop a reliable cytogenetic method for identification of *An. culicifacies* species complex which can be performed in field areas. Therefore it was attempted to identify the *An. culicifacies* sibling species complex by polytene chromosome method in the wild caught mosquito population at malaria endemic areas in Myanmar.

MATERIALS AND METHODS

Selection of study areas

The selection was based on the importance and representativeness of malaria endemic areas.

Field study areas

Gon-min-soe Village, Paukkaung Township, Bago Division and Se-daw-gyi Village, Madaya Township, Mandalay Division were selected for the collection of blood fed wild adult *An. culicifacies* mosquitoes to identify its sibling species complex by determining the ovarian nurse cell chromosome. Gon-min-soe Village, with the population of about 1500, lies in a valley on the Pyae-Paukkaung-Oktwin road (Mahar-buhar road). Most of the villagers are farmers, government timber staff and some are bamboo cutters. Se-daw-gyi Village is situated beside the Se-daw-gyi Canal and it is 3 miles away from Se-daw-gyi Dam, Madaya Township, Mandalay Division. About 2000 population are residing in the village and one primary school and one Rural Health Center (RHC) are situated in the village. Majority of the population are government staffs and dam workers, minority are farmers and fishermen.

Laboratory study

Cytotaxonomical study of all collected *An. culicifacies* samples from both Gon-min-soe and Se-daw-gyi villages was done in MERD, DMR (LM).

Study design

The combination of a field and laboratory-based study.

Mosquito sampling methods and samples

Adult mosquito collection

Adult mosquitoes were collected by several methods using cattle baited big net trap and hut collection, indoor and outdoor resting night collection, outdoor resting morning collection and morning indoor resting collection in the field.

Larval collection

Anopheles mosquitoes larvae were collected from sand pools in stream-bed, water pools in rocky place, canal bed, water pools in creeks which were within 3 kilometer radius around the study villages. Larvae were kept in standard chamber for emergence to adult.

Ovaries specimen collection

Ovaries of all semi-gravid *An. culicifacies* were dissected and separated out and kept in Cornoy's fixative (methanol and acetic acid mixture) in screw-capped bottles in field station. At the laboratory, the Cornoy's fixative fixed specimens were stored in refrigerator at 4°C for ovarian nurse cell identification within six months. Field collected other Anopheles mosquitoes were not dissected for ovarian nurse cell chromosome study but they were taken alive to the laboratory of MERD, DMR (LM) for colonization and taxonomical research works.

Cytogenetic method

Procedures

1. Wild caught adult mosquitoes and adult mosquitoes emerged from pupae collected during larval surveys were identified by the keys of Barraud [11],

Peyton and Scanlon, [12], Delfinado [13], Reid [14], Zavortink [15], Harrison and Scanlon [16], Payton and Harrison [17] and Harrison [18].

2. Blood fed *An. culicifacies* were kept in paper cups for ovarian development (Christopher stage III) for 12 hours, 24 hours and 36 hours, and then killed by chloroform vapours.
3. The ovaries were dissected and fixed in 1 part glacial acetic acid and 3 parts methanol or ethanol (Cornoy's fluid). After fixation, ovaries were kept for 36 hours at room temperature. (After this stage, specimens can be kept up to 6 months in 4°C).
4. Ovaries were softened in a drop of 50% propionic acid for a minute on a glass slide.
5. Propionic acid was removed and a few drops of diluted 2% lacto-aceto-orcein stain were added and kept for 10 minutes.
6. Tapping on the cover slip with a blunt ended tapper to obtain evenly spread chromosome.
7. After 3 minutes, slides were examined with Leica imaging system using IM50 software.
8. Polytene chromosomes were identified by chromosomal maps of sibling species A, B, C and D [9] and data were analyzed by Microsoft Excel.

RESULTS

Detail description of Anopheline mosquitoes collection is shown in Table 1. The table shows that a total of 4 anopheles species from Gon-min-soe Village and 10 anopheles species from Se-daw-gyi Village were caught in survey periods. Maximum number of *An. culicifacies* adult and larvae were collected in Gon-min-soe Village, followed by *An. maculatus* and *An. stephensi*. In Se-daw-gyi Village, maximum number of *An. vagus* and *An. annularis* were collected, followed by *An. phillipinensis* and *An. culicifacies*. Twenty-four *An. minimus* adults were

collected in cattle bait but *An. dirus* adults could not be collected from human and cattle baits and *An. dirus* larvae could not be collected in both areas. High numbers of *An. culicifacies* larvae were found in sand pool on the bed of Gon-min-soe Creek and Se-daw-gyi Canal.

Ovarian nurse cell development

After blood meal the fertilized eggs were developed in the ovary and at the same time different stages of ovarian nurse cell and chromosome were also developed. Figure 1 shows that 12 hours and 36 hours aged ovarian nurse cell and chromosome development were present in upper parts of the follicle in ovarian cell.

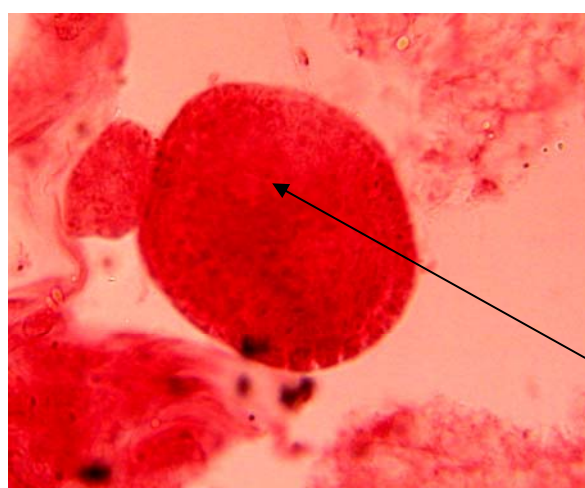
A total of 648 *An. culicifacies* from Bago and Mandalay Divisions were collected and examined for the ovarian nurse cell polytene chromosome analysis. Readable chromosomes were obtained from 216 specimens of 36 hours after blood meal groups. Up to 70-80% of specimens fixed in both ethanol and methanol fixatives showed clear chromosome bands (X and arm 2). Percent detection of chromosome bands were not significantly different between 12 hours, 24 hours and 36 hours aged ovaries and the outcome of methanol fixative and ethanol fixative was not statistically different. The chromosome detection rates are also not different between Bago and Mandalay Divisions. All 648 specimens were identified as *An. culicifacies* sibling species "B" when compared with the reference bands of X and arms 2 chromosome figures (Fig. 2 & 3). The chromosome of 36 hours aged ovaries was found to be highest and clearest chromosome bands in both areas (Fig. 4).

DISCUSSION

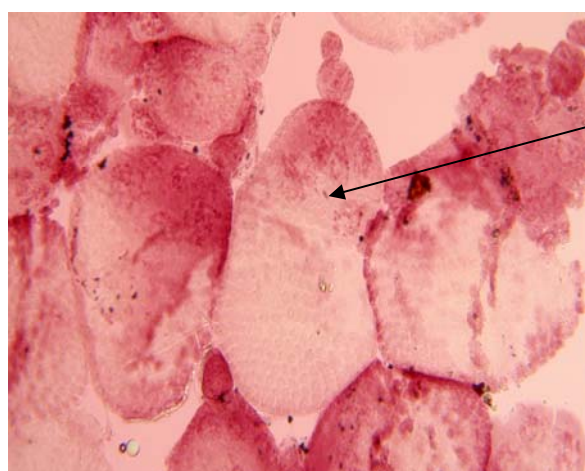
From the entomological aspect, *An. culicifacies* was found in high numbers during the study periods in both Gon-min-soe Village and Se-daw-gyi Village. *An. culicifacies* could not be assumed as the main vector of malaria since *An. minimus* may play a main vector of transmitting malaria in the region.

Table 1. Anopheline mosquitoes collection from two malaria endemic areas during survey

Anopheles species	Gonminsoe Village, Paukkaung Township, Bago Division				Saedawgyi Village, Madaya Township, Mandalay Division			
	Cattle bate	Morning collection	Night collection	Total number of collection	Cattle bate	Morning collection	Night collection	Total number of collection
<i>An. minimus</i>	-	-	-	-	24	-	-	24
<i>An. maculatus</i>	-	-	2	2	52	-	6	58
<i>An. annularis</i>	-	-	-	-	188	-	-	188
<i>An. phillipinensis</i>	-	-	-	-	120	-	-	120
<i>An. culicifacies</i>	21	136	456	613	30	5	-	35
<i>An. vagus</i>	3	4	14	21	151	23	15	189
<i>An. aconitus</i>	-	-	-	-	22	-	-	22
<i>An. tesslatus</i>	-	-	-	-	12	-	-	12
<i>An. stephensi</i>	8	2	28	38	8	-	-	8
<i>An. jamaasi</i>	-	-	-	-	10	-	-	10
Total no. of Anopheline mosquitoes collection	32	142	500	674	617	28	21	666



12 hours aged ovarian nurse cell and chromosome development in follicle



36 hours aged ovarian nurse cell and chromosome development in follicle

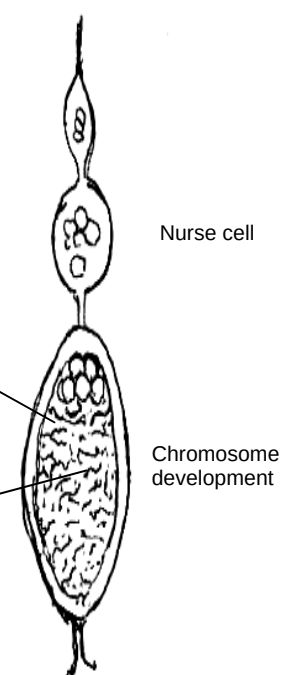


Fig. 1. Developmental stages of ovarian nurse cell and follicle in ovary



Fig. 2. Arm 2 chromosome after preparation of polytene chromosomes method of *An. culicifacies* sibling species B

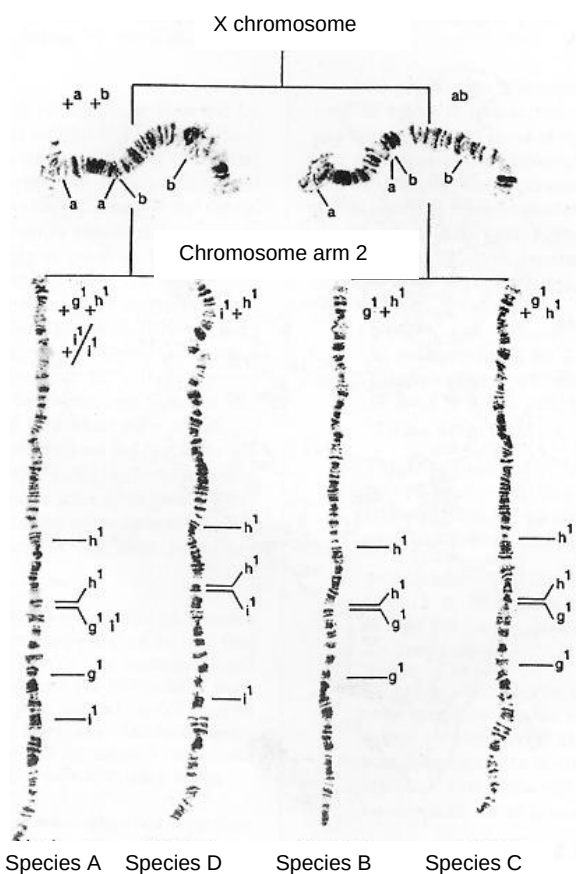


Fig. 3. Schematic representation of polytene chromosomes of *An. culicifacies* sibling species (Subbarao et al., 1988)

Anopheles dirus played secondary role due to its absence during the study period. Dissection reports in the past on *An. culicifacies* indicated that it was not a major vector of malaria in Union of Myanmar [1, 2] but laboratory feeding experiment

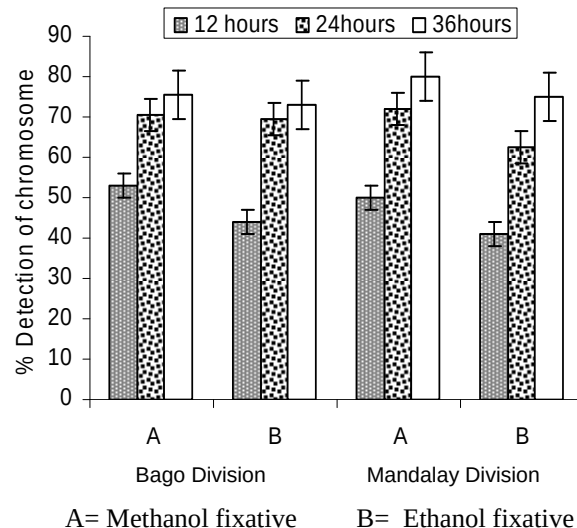


Fig. 4. Comparison of detectable chromosome with age specific ovaries fixed in methanol and ethanol fixatives

using *An. culicifacies* sibling species A and B mosquitoes in India and Pakistan showed that both species supported sporogony of *P. falciparum* and *P. vivax* [10, 19]. *An. culicifacies* type "B" was reported to be a main vector in Sri Lanka [20] but poor vector in India [9]. *An. culicifacies* is the major vector of malaria in rural areas of northern India [19] and also indicated that in northern India where *An. culicifacies* sibling species A and B are prevalent, salivary gland dissections of mosquitoes collected in a few villages around Delhi indicated that species A, with a sporozoite rate of 1.65%, is definitely a vector of malaria. However, the role of species B could not be established. *An. culicifacies* collection from Tha-bye-wa Village, Oktwin Township, Bago Division revealed that they are probable secondary vector but vector incrimination study confirmed *An. dirus* and *An. minimus* to be primary vectors of malaria, and positive for *P. falciparum* and *P. vivax* sporozoite antigen in their salivary gland by ELISA method [21, 22, 23].

Diluted 2% lacto-aceto-orcin stain is a very good stain for polytene chromosome staining and chromosome bands are clearly observed by microscopy. In cytotaxonomical study, 12 hours aged ovarian nurse

cell and chromosome were found weak and faint bands when stained with diluted 2% lacto-aceto-orcein stain but 24 and 36 hours aged had strong resolution and dark bands were observed but readable. Sharp and clear chromosomes were obtained from the specimens of more than 36 hours after blood meal group and it was good enough for ovarian nurse cell polytene chromosome study with clear identification of sibling species complex (Fig. 2). Other researchers revealed that ovaries up to Christopher stage III (after blood meals) were good for polytene chromosome study for *An. culicifacies* complex [5, 24]. Some 70-80% of specimens showed clear chromosome bands (X and arm 2) with fixed in both ethanol and methanol fixatives (Fig. 4). Both ethanol and methanol fixatives were suitable but methanol fixative have slight advantage over ethanol fixative for ovarian nurse cell polytene chromosome cytogenetic study.

Present study showed that type "B" species are abundant in Bago and Mandalay Divisions. The banding pattern was that of Xab which includes sibling species B and C. On the basis of the banding pattern on chromosome arm 2, all dissected samples of *An. culicifacies* were identified as sibling species B. Therefore *An. culicifacies* B is abundantly present in Paukkaung Township, Bago Division and Madaya Township, Mandalay Division. Ovarian nurse cell cytogenetic method was found to be accurate, simple and can be applied in the field situation. Although *An. culicifacies* is not a primary vector in Myanmar, they are found abundantly in malaria endemic area of foot-hill areas together with the primary vectors of *An. dirus* and *An. minimus*. Better understanding of species complex distribution and their role in malaria transmission will be beneficial for malaria prevention and control.

Conclusion

Ovaries of *An. culicifacies* developed after 36 hours blood meal provided best chromosome band in both ethanol and methanol fixatives. Present cytogenetic

method for identification of *An. culicifacies* complex was able to identify species complex "B". This information will be valuable in prevention and control of malaria. In malaria endemic areas, where *An. culicifacies* is present with other main vectors of malaria, polytene chromosome cytogenetic method could be used to identify sibling species complex. It is applicable for field based identification of anopheles vector sibling species. Application of polytene chromosome method should be encouraged and used as a tool for malaria prevention and control. Further studies are required to find out the presence of other type of species complexes in malaria endemic areas. Vectorial capacity of *An. culicifacies* type "B" should be studied in detail.

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Sensitivity and specificity of parasite lactate dehydrogenase (pLDH)-based rapid diagnostic test (SD Bioline malaria antigen test) in comparison with parasite density

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A cross-sectional hospital-based study (Thanbyuzayat Township Hospital, Mon State) on 103 patients, who were tested with malaria microscopy and parasite lactate dehydrogenase (pLDH)-based rapid diagnostic test strips (RDTs) during 2008. The blood film results indicated that 84 (81.55%) patients were malaria parasite positive and 19 (18.45%) were malaria negative. Forty-two cases diagnosed as *P. falciparum* by RDT were diagnosed by microscopy as 35 cases of *P. falciparum*, 2 cases of *P. malariae*, one case of *P. falciparum* and *P. malariae* mixed infection, and 4 cases of *P. falciparum* and *P. vivax* mixed infection. Forty-one patients diagnosed as non *P. falciparum* (*Plasmodium* species other than *P. falciparum*) by RDT were diagnosed by microscopy as 39 cases of *P. vivax* and 2 cases of mixed infection (*P. falciparum* and *P. vivax*). Twenty cases diagnosed as negative by RDT comprised of nineteen as negative and one as *P. vivax* by microscopy. The sensitivity was 100% and 97%, specificity was 90% and 96%, positive predictive value was 83% and 95% and negative predictive value was 100% and 98% for *P. falciparum* and non *P. falciparum*, respectively. The lowest parasite density detected by test was 109.5 parasites/ μ l for *P. falciparum* and 160 parasites/ μ l for *P. vivax*. In this study, the band intensity was significantly related to parasite density and the differences between mean parasite count was significant between faint and intermediate, intermediate and strong. Thus, it can generally estimate the parasite count and can be used in the field for screening of malaria parasite for preparation of quality control sample.

INTRODUCTION

Malaria is the most important parasitic disease of man and remains a major health problem in tropical countries. It is a disease caused by a protozoan of the genus *Plasmodium* namely: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*. At present 1326 million people or 83% of population in South East Asia (SEA) are at risk of malaria. The region harbours two main malaria parasites, *Plasmodium falciparum*, which is a well-known killer and *Plasmodium vivax* which causes relapse episodes of malaria [1].

About 95% of the population of moderate to high risk of malaria in SEA region is living in Myanmar, Thailand, India and Indonesia.

Multidrug-resistant *Plasmodium falciparum* is highly prevalent on the Thai-Cambodia and Thai-Myanmar border areas and Myanmar contributes to around 65% of reported malaria deaths [2]. In 2006, the leading causes of morbidity and mortality due to malaria were 7.1 per cent and 9 per cent, respectively [3]. The recommended strategy to control malaria is based on prompt and accurate diagnosis and the use of an artemisinin combination therapy (ACT) [4].

An ideal diagnostic test for malaria must be simple, inexpensive, be able to be performed rapidly, easy to interpret and discriminate between *P. falciparum* and the other malarial species. Microscopic detection of malaria parasites in thick and/or thin blood smears remains the most appropriate diagnostic method in endemic countries [5]. However, microscopy needs well-qualified personnel and also time-consuming and facilities are not always available in endemic areas. An alternative to the classical thick/ thin blood smear and microscopic examination is the Rapid Diagnostic Test (RDT) which is based on the detection of antigens derived from malaria parasites using immunochromatographic methods. They are available as dipsticks, cards and cassettes and can be easily taken into the field and particularly useful where the electricity supply is non-existent. There are two major classes of RDTs that detect *P. falciparum* only and those that detect *P. falciparum* plus one or more other species of malaria. The RDTs can also detect parasites which are sequestered in the deep vascular compartment whereas peripheral blood smears cannot detect.

The WHO recommendation is that a RDT should have a sensitivity of $\geq 95\%$ at a level of ≥ 100 parasites/ μl and should have at least as accurate as results derived from microscopy [5]. RDTs have now been integrated into routine practice in several national malaria control programmes (e.g. Thailand, Cambodia and South Africa) [4]. The objective of National Malaria Control Programme of Myanmar is to reduce malaria morbidity and mortality mainly through increasing accessibility to quality diagnosis and appropriate treatment [3]. In this connection, studying the relationship between parasite density and band intensity of available RDTs might assist to improve evidence-based clinical management of malaria.

Objectives

- To determine the sensitivity and specificity of pLDH-based RT (SDD

Bioline Malaria Antigen Test).

- To determine the parasite density in patients attended the out-patient department (OPD)
- To compare the band intensity of RDT with parasite density.

MATERIALS AND METHODS

Thanbyuzayat Township, Mon State was selected for cross-sectional hospital-based study from January to October, 2008.

Sample size

A minimum sample required was 100 subjects by assuming that prevalence of clinically suspected malaria patients being 0.5 (conservative estimate) at 95% confidence interval and 10% marginal error [6].

Inclusion criteria

All patients with uncomplicated malaria, aged 6 years and above were included. Axillary temperature of $\geq 37.5^\circ\text{C}$ or history of fever during the previous 48 hours. Informed consent provided by patients or parents.

Exclusion criteria

- Presence of one or more danger signs such as inability to drink or repeated vomiting, recent history of convulsions, lethargy or unconsciousness, inability to sit or stand up, or any sign of severe or complicated malaria
- Presence of pregnancy, lactation
- Presence of underlying diseases (e.g. cardiac, renal, hepatic diseases)

In the study township, 10% Giemsa-stained thick and thin blood films were examined independently by two microscopists (the researcher and an experienced microscopist who was not aware of test results). Parasite densities were measured by counting asexual parasites (rings, trophozoites and schizonts) against 300 WBCs in the thick film. Parasitaemia was calculated as follows [7]:

$$\text{Parasite density}/\mu\text{l} = \frac{\text{number of parasites counted} \times 6000}{\text{number of leucocytes counted}}$$

A blood film was recorded as negative after examining 200 thick blood smear fields. All slides showing >20% discrepancies were excluded from this study. All blood samples were also tested with the RDTs according to manufacturer's instructions.

Interpretation of the test:

The test uses two polyclonal antibodies which have been immobilized as two separate lines across a test strip. One antibody (test line 1) is specific to *P. falciparum*. The other antibody (test line 2) is specific to the four *Plasmodium* species (*falciparum*, *vivax*, *malariae*, *ovale*) (Fig.1). Besides, LDH of *P. falciparum* (control line) is immobilized for the procedural control.

The intensity of band was graded as follows:

- 0= no band (negative)
- 1+= faint band, but clearly visible (positive)
- 2+= medium intensity band, stronger than 1+ but less than control band (positive)
- 3+= equal or stronger intensity than the control band (positive) [8]

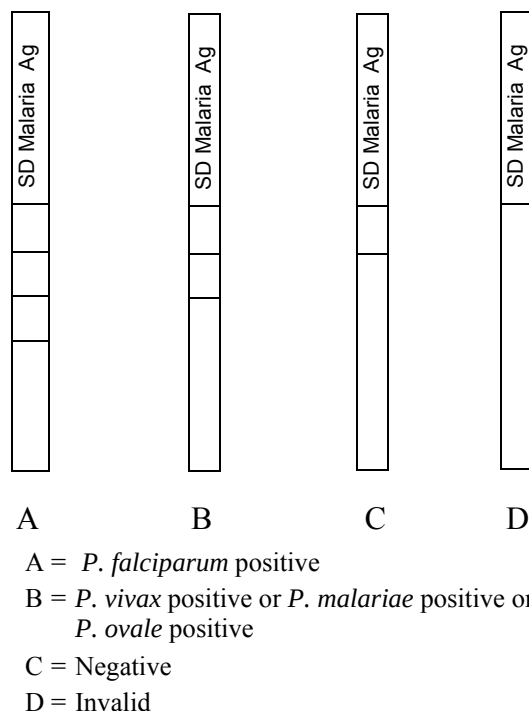


Fig. 1. SD Bioline Malaria Antigen dipstick (RDT)

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated by

Confidence Interval Analysis Software Version 2.0. The correlations between the parasite densities and band intensity were calculated by SPSS version 11.5. Mixed infection cases were excluded by microscopy. A total of 103 blood samples were tested by pLDH test and their results were compared with the results of blood films examined.

RESULTS

During one-year study period, 103 clinically suspected malaria patients attending the outpatient clinics of Thanbyuzayat, Mon State were screened for malaria. Around 77% were male patients and 23% were female patients. The age range was 6-58 years. The high incidence was noted in age group between 20 to 29 years. In which, 80 patients (77.67%) were rubber plantation laborers.

Comparison between microscopy and RDTs

When compared with RDT results, 42 cases diagnosed as *P. falciparum* were diagnosed by microscopy as *P. falciparum* 35, *P. malariae* 2, *P. falciparum* and *P. malariae* 1, *P. falciparum* and *P. vivax* 4 cases. Forty-one patients diagnosed as non *P. falciparum* (*Plasmodium* species other than *P. falciparum*) by RDT were diagnosed by microscopy as *P. vivax* 39 and *P. falciparum* and *P. vivax* 2 cases. Twenty cases diagnosed as negative by RDT also have 19 as negative and 1 as *P. vivax* by microscopy. Two cases diagnosed as *P. falciparum* by RDTs were *P. malariae* infection by microscopy. One patient that was diagnosed as *P. vivax* by microscopy (i.e. parasite density 166 parasites/ μ l) was not detected by pLDH-based RDTs. The lowest intensity that was detected by RDT was 109.5 parasites/ μ l for *P. falciparum* and 160 parasites/ μ l for *P. vivax*.

Sensitivity and specificity of RDT

For *P. falciparum*, the sensitivity was 100% (95% CI = 90-100), the specificity was 90% (95% CI=80-95), the PPV was 83% (95% CI=69-92) and the NPV was 100% (95%

CI=94-100). The Kappa value was 0.86 and 95% CI was 75-96.

For non *P. falciparum*, the sensitivity was 97% (95% CI=87-100), the specificity was 96% (95% CI=89-99), the PPV was 95% (95% CI=84-99) and the NPV was 98% (95% CI=91-100). (The Kappa value=0.94 and 95% CI =87-100). All RDT test showed positive control line.

Band intensity and parasite density

For *P. falciparum*, the minimum parasite density that was scored as 1+ (faint) band intensity (in RDT test) was 416.6 parasites/ μ l and the maximum was 7685.8 parasites/ μ l, mean value was 4051.19. In 2+ (medium) band intensity, the minimum was 4116 parasites/ μ l and the maximum was 13182.2 parasites/ μ l, mean value was 8649.09. In 3+ (strong) band intensity, the minimum parasite density was 16513.3 parasites/ μ l and the maximum was 49304.1 parasites/ μ l, the mean value was 32908.7 (Figure 2).

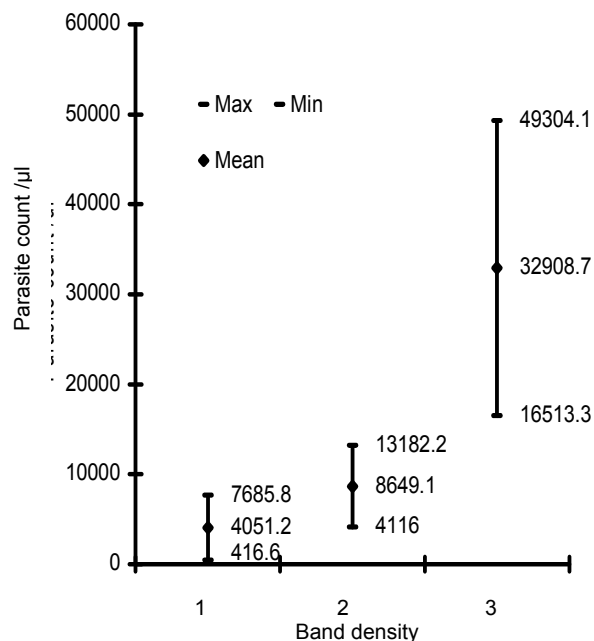


Fig. 2. Mean *P. falciparum* parasite count in different band intensity

For non *P. falciparum*, the minimum parasite density detected in 1+ (faint) band intensity of SD Bioline Malaria Antigen test was 160 parasites/ μ l and the maximum was

600 parasites/ μ l, the mean value was 378.90. In 2+ (medium) band intensity, the minimum was 2601.1 parasites/ μ l and the maximum was 7332.7 parasites/ μ l, mean value was 4966.86. In 3+ (strong) band intensity, the minimum was 6031.1 parasites/ μ l and the maximum was 17552.3 parasites/ μ l, mean value was 11791.75 (Fig. 3).

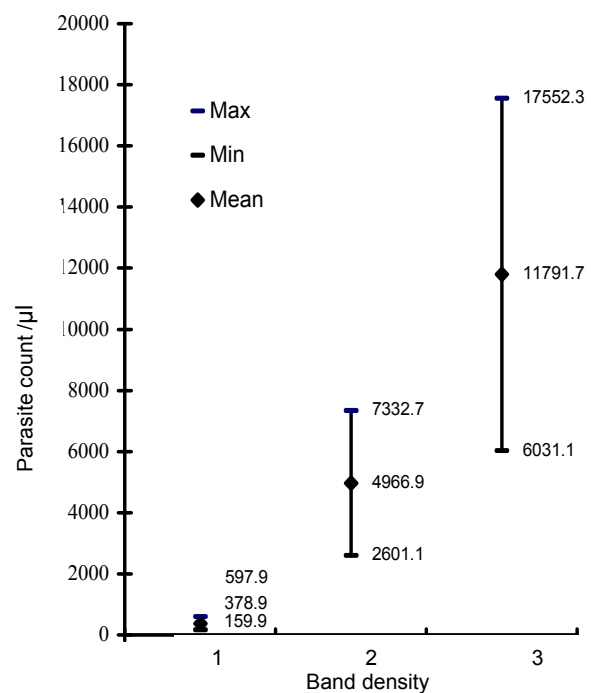


Fig. 3. Mean non *P. falciparum* parasite count in different band intensity

Twelve slides showing more than 20% discrepancies and seven slides with mixed infections were excluded. The lowest parasite density that was also detected by the RDT was 109.5 parasites/ μ l for *P. falciparum* and 160 parasites/ μ l for *P. vivax*.

The differences in mean parasitemia between faint and intermediate, and intermediate and strong were significant for *P. falciparum* ($P=0.0005$ and $P=0.0005$, respectively) than for non *P. falciparum* ($P=0.011$ and $P=0.001$, respectively).

Correlation of band intensity with parasite density

The correlation between band intensity and parasite density of non *P. falciparum* was significant ($R = 0.61$, $P = 0.01$) (Fig. 4) and that of *P. falciparum* was also significant ($R = 0.66$, $P = 0.01$) (Fig. 5).

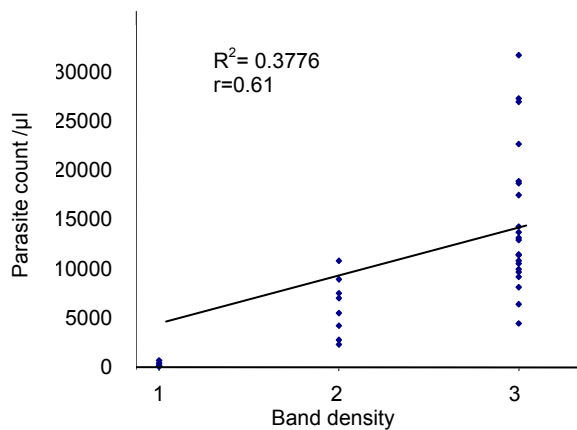


Fig. 4. Correlation between band intensity and parasite count in non-falciparum malaria infection

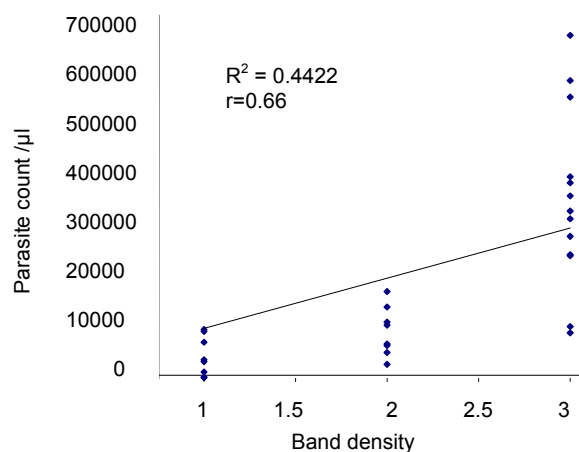


Fig. 5. Correlation between band intensity and parasite count in falciparum malaria infection

DISCUSSION

The resurgence of malaria has renewed interest in many countries and malaria caused by *Plasmodium falciparum* is one of the leading causes of morbidity and mortality in tropical countries. It is one of the priority public health problems in Myanmar. With the introduction of new more costly anti-malarial drugs in Artemisinin combination therapy, confirmation of malaria infection before initiating treatment is essential. Several methods have been developed to supplement and replace the conventional microscopic method. The most promising new malaria diagnostics are the immunochromatographic rapid diagnostic tests.

The current market price of a rapid diagnostic test in developing countries is about U.S\$ 1.55-3.5 (depending on the number of targeted species and quantity), compared with microscopy at U.S\$0.12-0.4 per malaria smear [9]. But in this study, the price of RDT test was 1,200 kyats per test which was not so expensive compared to other RDT and also easily available. Although the cost of microscopy was not expensive, it was needed to maintain the equipment (e.g. reagent, microscopy, electricity, etc) which was really expensive. The cost of artemisinin combination treatments (ACTs) for negative patients (over-diagnosis) could balance the extra cost of using rapid diagnostic tests. Prompt and accurate diagnosis will not only improve malaria treatment but also possibly reduce morbidity due to other febrile illnesses.

Microscopy is considered as the gold standard for malaria diagnosis and treatment but it has also disadvantages. It requires well-trained microscopists and regular maintenance of functional infrastructures. Moreover, the chance of false positive results increases with poor blood film preparation (i.e. artifacts commonly mistaken for malaria parasite) and normal blood components (e.g. platelet). The chance of false negative results increases with decreasing parasite densities. Errors can occur in differentiating between *P. vivax* and *P. ovale*, *P. falciparum* and *P. malariae*. However, in this study, *P. ovale* infection was not detected. Underreporting of mixed species infections is also common when the number of parasite density of one type of species involvement is very low.

In this study, the lowest parasite density that was also detected by RDT test was 109.5 parasites/µl for *P. falciparum* and 160 parasites/µl for *P. vivax* that the RDT test is as sensitive as thick film microscopy in the diagnosis of malaria. But the test assay cannot distinguish *P. falciparum* mono-infection from mixed infections (*P. falciparum* and other species) because one of the isozymes of pLDH specific antibody for

P. falciparum had same migrating distance as non *falciparum* bands [10]. Therefore, mixed infections were indistinguishable from *P. falciparum* in this assay. Therefore, false positive results were observed. In two patients, the RDT test had come into view as *P. falciparum* but blood film showed *P. malariae* only, the underlying reason was not known. The RDT test also gave a false negative result for only one patient (i.e. *P. vivax* diagnosed by blood film) who had an estimated parasite densities of 166 parasites/ μ l which was near to the lowest count of this test strip in this study. This suggests that parasite density can also affect the sensitivity of the RDT test. It is possible that low levels of parasitemia in those patients correspond to low pLDH blood concentration, resulting in failure to capture the antigen by the antibody present in the dipstick. However, the sensitivity of the test was very close to that of the microscopic examination of blood smears i.e. for *P. falciparum* the test showed 100% sensitivity and 90% specificity and for non *P. falciparum*, the sensitivity was 97% and the specificity was 96% and it did not require highly skilled personnel to perform or interpret results. The test has the added advantage that it can detect all four *Plasmodium* species and can be used to follow the efficacy of the drug therapy since it detects an enzyme produced only by live parasites [11].

Another objective of this study was to compare the band intensity of rapid diagnostic tests with parasite density. Twelve slides checked by two microscopists showing more than 20% discrepancies and 7 mixed infections were excluded from this study to get any correlation between band intensity and parasite density. The minimum and maximum parasite densities were 160 parasites/ μ l and 7685.8 parasites/ μ l (i.e. score as 1+), 2601.1 parasites/ μ l and 13182.2 parasites/ μ l (i.e. score as 2+), 6031.1 parasites/ μ l and 49304.1 parasites/ μ l (i.e. score as 3+), respectively. The differences in mean parasitemia between faint and intermediate, and intermediate and

strong were significant for *P. falciparum* ($P=0.0005$ and $P=0.0005$, respectively) and also for *P. vivax* ($P=0.011$ and $P=0.001$, respectively). The correlation between band intensity and parasite density of *P. falciparum* was $R^2=0.4422$, $r=0.66$, $P=0.012$ and non *P. falciparum* was $R^2=0.3776$, $r=0.61$, $P=0.012$ (Bivariate, two tailed, Pearson correlation, by SPSS 11.5).

Therefore, this test can be used in the field for screening of malaria parasite and also for preparation of quality control sample to ensure adequate performance of the RDT. If this observation is validated in larger multi-center clinical studies, the pLDH dipstick may prove to be a reliable alternative for the diagnosis of *P. falciparum* or non *P. falciparum* malaria. In contrast to dipsticks based on the histidine rich proteins, the pLDH assay measures the enzyme levels of intact viable parasites and so can be used to monitor response to specific treatment [11]. It may be a valuable adjunct to clinical assessment of the patient and blood film microscopy. However, inability to distinguish mixed infection from *P. falciparum* with this dipstick remains a problem in clinical utility. Finally, its final resolution must await a non *falciparum* specific antibody that does not cross-react with *P. falciparum* and the RDTs should be easily feasible in public especially in remote areas where malaria is common.

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Knowledge, attitude and practice on HIV/AIDS among the highway coach staff in Aung Mingalar Highway Coach Station

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A cross-sectional descriptive study was carried out in 2009 to assess the knowledge, attitude and practice on HIV/AIDS among the highway coach staff in Aung Mingalar Highway Coach Station in Yangon. A total of 286 highway coach staff was interviewed using a pre-tested structured questionnaire. Fifty-seven percent of the respondents could identify the modes of transmission and prevention methods regarding HIV/AIDS. However, there were still misconceptions and wrong beliefs concerning the disease transmission and prevention methods. Condom knowledge among the highway coach staff was high. Fifty-one percent of the respondents in the study had positive attitude towards HIV infected persons and preventive measures. The condom use rates with the sexual partner during the last three months were 85% and 88% for Casual Acquaintance (CA) and Commercial Sex Worker (CSW), respectively. There are statistically significant associations between sexual practice (having sex with a CSW during the last 3 months) and marital status, indicating that unmarried respondents were more likely to take the risk behaviour than married respondents, and with being away from home for more than 2 months meaning that the longer the duration away from home, the higher the risk of practicing the risky sexual behaviour.

INTRODUCTION

The human immunodeficiency virus (HIV), causing acquired immunodeficiency syndrome (AIDS), is an unprecedented public health emergency, having already caused enormous ill health and mortality worldwide. Given the scale of the epidemic, AIDS is now considered not only a health problem, but also a developmental and security threat. Three decades into the epidemic, there is still no vaccine and cure. There is, however, considerably more information available on how the virus is spread, as well as an increased understanding about prevention strategies and what constitutes effective treatment and care. The social and economic conditions that facilitate the spread of HIV are also well understood. Despite this, risk behaviours and risk environment continue to persist, and HIV continues to spread among

individuals and across national and regional borders.

HIV/AIDS problem has been recognized as one of the major public health problems in Myanmar. Key population at high risk and vulnerability included sex workers, clients of sex workers, drug users, men who had sex with men, and partners of people living with HIV. Thus, most of the prevention and surveillance activities aim at targeting the most at risk. However, high priority is also given to populations vulnerable to risk which include institutionalized populations, mobile population and young people.

Highway coach staff (highway bus drivers and their assistants) represent one of the key bridge population groups between high and low risk communities. The nature of their work and the stress associated with long periods of travel and being away from family for prolonged stretches of time can

result in the drivers and spares indulging in risky behaviours with regard to HIV/AIDS.

The floating population of transport workers is one of the major sources of infection to the low risk partners which are mostly their life time partners who are naive about disease transmission. Thus, by understanding the knowledge, attitude and the risk taking behaviour of the highway coach staff, preventive options and health educational input can be provided to the National AIDS programme for better development of strategies aiming towards the bridging population.

General objective

- To assess the knowledge, attitude and practice on HIV/AIDS among the highway coach staff in Aung Mingalar Highway Coach Station.

Specific objectives

- To identify the socio-demographic characteristics of the highway coach staff in Aung Mingalar Highway Coach Station
- To determine the levels of knowledge, attitude and practice on HIV/AIDS among the highway coach staff in Aung Mingalar Highway Coach Station
- To find out the association between the socio-demographic characteristics and (i) knowledge scores, (ii) sexual practice of the highway coach staff in Aung Mingalar Highway Coach Station

MATERIALS AND METHODS

Study design

Cross-sectional descriptive study

Study population

Highway coach staff (drivers and spares) in Aung Mingalar Highway Coach Station

Study area

Aung Mingalar Highway Coach Station, Yangon

172

Study period

July 2009 to October 2009

Sample size

A total of 286 coach staff were included in the study.

Sampling method and procedure

Highway coach staff are mobile persons who come into highway station only during their assigned duty. So, consecutive sampling was used until the required number of sample size was obtained.

Data collection tool and technique

A pre-tested face-to-face interview questionnaire was used for data collection. Before data collection, pre-test was done with twenty drivers and spares in highway coach station and corrected some of the questions. Before asking the attitude questions, the Likert scales card was distributed to the respondent and described him how to answer the attitude questions. In this study, 286 highway coach staff were participated to reduce drop-outs during data collection. But, drop-outs were not seen and data from all respondents were used for analysis. After obtaining informed consent, information concerning the highway coach staff's socio-demographic, knowledge, attitude and practice on HIV/AIDS was obtained in a private room.

Data analysis

All the collected data were checked for completeness and consistency on a daily basis by the investigator before leaving the data collection site. Data entry was done by using Epi data software version 3.1. After data entry, data cleaning was done by checking frequency distribution tables, graphs, outliers and errors. Analyzing of the data was done by using SPSS 16.0 version. The association for categorical data was calculated using the Chi square statistical test.

Ethical consideration

Informed consent was obtained from all the interviewees and also from the supervisory groups. Ethical clearance was obtained from

the ethical board and Post-graduate Board of Studies.

RESULTS

Socio-demographic characteristics of the highway coach staff in Aung Mingalar Highway Coach Station

A total of 286 highway coach staff participated in this study. Among them, 144 drivers and 142 spares were included. About 75% of the highway coach staff were from the ages of 25 to 49 years. Their mean age (years) was 33.12 ± 9.275 SD with a minimum age of 18 years and maximum age of 59 years. About 72% of the respondents had middle and high school level of education, 32% were singles, 63% received income of kyats 100,000 and over per month, 46% had over five years of service, and 37% were away from home for more than one week.

Knowledge on HIV/AIDS among the highway coach staff in Aung Mingalar Highway Coach Station

Although most of the respondents had correct knowledge regarding major mode of transmission, there were still misconceptions regarding HIV transmission. About 27% of the respondents stated that HIV can be transmitted by mosquito bite and 12% mentioned that eating or drinking together with an HIV infected person can transmit the disease. Eighty-two percent said that being faithful to an uninfected life-time partner could prevent the transmission of the disease. Using condom in every sexual act with unknown partners was correctly answered by 74% of the respondents. Nearly 60% of the respondents knew that avoidance of unnecessary injection was a preventable method. Mean knowledge score of highway coach staff in Aung Mingalar Highway Coach Station was 12 with standard deviation of 2. The median knowledge score was 12 and maximum and minimum were 2 and 15, respectively. A total of 15 points were given and who obtained 12 marks and above were categorized as high and below 12 marks were categorized as low. Among

the 286 respondents, 164 (57.3%) obtained high knowledge score and 122 (42.7%) had low knowledge score (Table 1).

Table 1. Knowledge on HIV/AIDS among the highway coach staff in Aung Mingalar Highway Coach Station

Variables	Correct	Incorrect	Don't know	Total
	No. (%)	No. (%)	No. (%)	No. (%)
Knowledge on mode of transmission of HIV/AIDS				
Unprotected sex	267 (93.4)	10 (3.5)	9 (3.1)	286 (100)
Mosquito bite	182 (63.6)	78 (27.3)	26 (9.1)	
Unscreened blood trans- fusion	273 (95.5)	5 (1.7)	8 (2.8)	
Eating or drinking	248 (86.7)	36 (12.6)	2 (0.7)	
Mother to fetus	252 (88.1)	21 (7.3)	13 (4.5)	
Tattooing	267 (93.4)	14 (4.9)	5 (1.7)	
Knowledge on prevention methods of HIV/AIDS				
Abstained from sex	186 (65)	93 (32.5)	7 (2.4)	286 (100)
Unnecessary injection	171 (59.8)	106 (37.1)	9 (3.1)	
Using condom	212 (74.1)	67 (23.4)	7 (2.4)	
Being faithful to partner	235 (82.2)	47 (16.4)	4 (1.4)	

Attitude on HIV/AIDS among the highway coach staff in Aung Mingalar Highway Coach Station

Although 37.1 percent of highway coach staff strongly agreed that they were willing to work together with HIV-positive persons, 12.9 percent strongly disagreed that they were willing to buy and use goods and foods from sellers who is HIV-positive. Over half of the respondents agreed eating or drinking together with HIV-positive person and 6.6% disagreed that they were willing to take care of AIDS patient in their family. Twenty-seven percent of all respondents said that they strongly agreed that condom must be used with regular partner and lover and only 1% strongly disagreed on keeping the condom to prevent HIV infection. About 20 percent of the respondents disagreed that a healthy and handsome man or a beautiful girl can be HIV- positive (Table 2).

Table 2. Attitude on HIV/AIDS among highway coach staff in Aung Mingalar Highway Coach Station

Variables	Strong-ly agree (%)	Agree (%)	Neutral (%)	Dis-agree (%)	Strong-ly dis-agree (%)	Total (%)
I am willing to work with an HIV-positive person	106 (37.10)	117 (40.90)	4 (1.400)	42 (14.70)	17 (5.90)	286 (100)
I will buy and use goods and food from seller who is HIV-positive	36 (12.60)	109 (38.10)	10 (3.50)	94 (32.90)	37 (12.90)	
I will eat together with a person who is HIV-positive	59 (20.60)	155 (54.20)	3 (1.00)	51 (17.80)	18 (6.30)	
I am willing to provide care to AIDS patient	146 (51.00)	113 (39.50)	2 (0.70)	19 (6.60)	6 (2.10)	
Condom must be used with a regular partner and lover	78 (27.30)	150 (52.40)	7 (2.40)	46 (16.10)	5 (1.70)	
Keeping a condom prevents HIV infection	99 (34.60)	138 (48.30)	8 (2.80)	38 (13.30)	3 (1.00)	
A healthy looking beautiful girl may be HIV positive	67 (23.40)	118 (41.30)	39 (13.60)	52 (18.20)	10 (3.50)	
Tell someone if I have HIV infection	67 (23.40)	140 (49)	4 (1.40)	54 (18.90)	21 (7.30)	
I can be at risk of HIV infection	23 (8.00)	89 (31.10)	32 (11.20)	110 (38.50)	32 (11.2)	
Being away from family is high risk of HIV infection	68 (23.80)	149 (52.10)	15 (5.20)	45 (15.70)	9 (3.10)	

Sexual intercourse and condom use within last three months among highway coach staff in Aung Mingalar Highway Coach Station

Among 286 highway coach staff, 61 (21.3%) had sexual intercourse with casual

Table 3. Sexual intercourse and condom use within last three months among the highway coach staff in Aung Mingalar Highway Coach Station (n=286)

Having sex within last three months	No.	Percent
<i>Having sex with a casual acquaintance (CA)</i>		
Yes	61	21.3
No	225	78.7
<i>Use of condom during the last sex with CA</i>		
Yes	52	85.2
No	9	14.8
<i>Having sex with a Commercial Sex Worker (CSW)</i>		
Yes	62	21.7
No	224	78.3
<i>Use of condom during the last sex with CSW</i>		
Yes	55	88.7
No	7	12.3

acquaintances within last three months. Among the respondents who had sexual intercourse with casual acquaintance within last three months, 85.2% had used condom. About 22% had sexual intercourse with a CSW within last three months, and among them, about 89% had used condom (Table 3). Among 92 single respondents, 32.6% of them had history of sexual intercourse with commercial sex worker within last three months and 93.3% of them used condom at last act.

The highway coach staff who gave history of sexual intercourse with CSWs within last 3 months were mostly found in 18 to 24 years age group and education level of primary school and below. Majority of them were spares, got monthly average income of less than 100,000 kyats and had 5 years and lower duration of service. There was a statistically significant association between marital status and practice. Therefore, unmarried respondents were more likely to take the risk behaviour than married respondents. Since age was also statistically significant, stratified analysis was carried out to see the effect of confounding on marriage, single older highway drivers (above 25 years) were more likely to practice risky sexual behaviour compared to their same age married

counterparts. There was also a statistically significant association between age, being away from home and sexual practice meaning that the longer the duration away from home, the higher the risk of practicing the risky sexual behaviour (Table 4).

Table 4. Association between sociodemographic characteristics and practice (history of sexual intercourse with CSWs within last three months)

Socio-demographic characteristics		Practice		Chi square & P value
		Yes (%)	No (%)	
Age	18-24 years	19(32.2)	40(67.8)	$\chi^2=4.85$
	25 years and above	43(18.94)	184(81.05)	$p=0.02$
Education level	Primary and below	8(26.7)	22(73.3)	$\chi^2=0.75$
	Middle	21(22.8)	71(77.2)	$p=0.69$
	High and above	33(20.1)	131(79.9)	
Job category	Spares	36(25.4)	106(74.6)	$\chi^2=2.24$
	Drivers	26(18.1)	118(81.9)	$p=0.13$
Monthly average income (Kyats)	<100,000	19(18.1)	86(81.9)	$\chi^2=1.26$
	≥100,000	43(23.8)	138(76.2)	$p=0.26$
Duration of service in the present job	≤5 years	35(22.4)	121(77.6)	$\chi^2=0.12$
	>5 years	27(20.8)	03(79.2)	$p=0.73$
Marital status	Unmarried	31(32)	66(68)	$\chi^2=9.14$
	Married	31(16.4)	153(83.6)	$p=0.003$
Being away from home	<1week	36 (20)	144(80)	$\chi^2=6.71$
	1 week to 2 months	14(18.4)	62(81.6)	$p=0.035$
	> 2 months	12(40)	18(60)	

DISCUSSION

Transport workers are predominately vulnerable to HIV/AIDS. This is because of the nature of their work. In particular the fact that they spend so much times away from home in situations favoring to high risk behaviours. As migrations become more prominent in the developing countries, the risk for transmission of HIV/AIDS also rises among the transport workers. HIV/AIDS is mainly affected to sexually active age groups and destroys the productivity of the country.

As in other studies, majority of the highway coach staff in Aung Mingalar Highway Coach Station were in their sexually active reproductive age years [1, 2]. Since their income seems to be quite well and some were unmarried, the risk taking behaviour towards the HIV/AIDS can be said to be favorable.

Although knowledge on HIV/AIDS was found to be high in this study, there were still misconceptions concerning the detail information of the disease. These findings are also similar to other studies in which general information of HIV are knowledgeable by drivers [2, 3].

Knowledge on preventive method due to sexual mode of transmission revealed that 65% of the respondents knew that abstinence from sexual activity was a preventive method. Eighty-two percent said that being faithful to partner was a preventive behaviour. Using condom was also known as preventive method by two-third of the respondents. These findings are similar to the study in Pune Ahmednagar Highway in India [4] in which knowledge of transmission by heterosexual route was much higher compared to knowledge of other routes of transmission.

Over fifty-seven percent of the respondents had above mean knowledge score. This finding highlights that highway coach staff in Aung Mingalar Highway Coach Station, in general, had good knowledge regarding HIV/AIDS. However, since there are still misconceptions concerning mode of transmission, HIV/AIDS education programs should focus on clear and precise messages concerning mode of transmission and prevention methods.

As an overall, the highway coach staff of Aung Mingalar Highway Coach Station had positive attitude towards HIV-positive persons, and can be considered much higher than the study done by Than Win [2].

Attitude towards risk of infection showed that nearly fifty percent of the respondents felt that they were not at risk. Similar

findings were seen in a study mentioning that most of the respondents did not think they could have HIV infection and did not want to say to other person if they were infected with HIV [2].

Seventy-five percent of the highway coach staff in this study agreed that being away from family was the high risk for HIV infection. A survey conducted by ITF in Uganda [5, 6] showed that 70 percent of the drivers had spent less than a week at home in the previous four months, found lovers and visited CSWs in several different cities along the routes they traveled. Sometimes, the drivers gave a lift to women in exchange for sex. Thus, being away from home is considered as the risk for engaging in behaviors that are likely to transmit the HIV infection.

Premarital sexual behavior was seen among 70.3% of the highway coach staff in this study. Among the 92 single respondents, 72.8% already had sex at the time of the study. Regarding the types of sexual partner, CSW (89.6%), CA (80.6%) and lover (59.7%) were found to be the most common types of partners for premarital sex. This finding pointed out that sexual exposure before marriage was quite high. This finding was higher than the rate observed by San Hla Mu *et al.* in 1993 among the highway drivers in which 42% of the unmarried persons had premarital sexual exposure [3].

In this study, 21% had sexual intercourse with a CSW or CA within the last 3 months. The condom use rates with the sexual partner during the last three months were 85% and 88% for CA and CSW, respectively. The condom use rate in this study was much higher to the study on taxi drivers in 2005 by Than Win which mentioned that condom use among taxi drivers was not satisfactory [2]. The higher condom used rate may be explained by the fact that “100 percent condom promotion activity” has been launched in Aung Mingalar Highway Coach Station in 2009.

There was a statistically significant association between sexual practice (having sex with a CSW during the last 3 months) and marital status indicating that unmarried respondents were more likely to take the risk behaviour than married respondents. There was also a statistically significant association between being away from home and sexual practice meaning that the longer the duration away from home, the higher the risk of practicing a risky sexual behavior.

Based on the study findings, the authors recommend to promote accessibility and availability of clear and precise messages concerning mode of transmission, disease process and disease prevention; and to implement regular refreshment educational training on HIV/AIDS since the population is mobile for highway coach staff.

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Socio-behavioral study on malaria prevention and control among forest related workers in hard-to-reach areas

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This study was done to determine the socio-behavioral factors on malaria prevention and control among the forest related workers in hard-to-reach areas of Pyin Oo Lwin Township. Community-based cross-sectional survey was carried out by collecting data on socio-demographic characteristics, knowledge, practice and behaviors of forest related workers using both quantitative and qualitative approaches. Study population consisted of people who were working at the forest related jobs in hard-to-reach areas of the township. A total of 204 workers were interviewed using pre-tested, closed-ended questionnaire. Twenty males and ten females of forest related workers were discussed at three focus group discussion (FGD) sessions. Among 204 interviewees, 149 (73%) had history of malaria and only 55 (27%) were free from malaria during the last two years. A total of 141 workers did not use the bed net. The old aged persons of frequent living in the forest without using bed net were having greater risks for getting malaria infection than the bed net users ($p=0.001$). Their reasons for not using bed nets were unaffordable condition and inconvenience for hanging of bed nets in the forest. According to the recommendations, the forest related workers should be supplied with specific kind of bed nets which are convenient for using in the forested sites.

INTRODUCTION

In Myanmar, malaria is the major health problem, like other developing countries in the world. About 20% of the people in Myanmar live in the malaria endemic forested areas. Moreover, migrating workers are gathered in the developmental projects located in forested sites, like construction of road and dam, mining of gold and gem and so on [1]. The migrant population needs to use preventive activities, such as carrying and using of bed nets. The most common reason for not having a bed net is the lack of money to buy one [2]. Many migrant workers sleep in the forest without bed nets. Only small, thin blankets, such as sarongs, are used to protect their bodies from mosquitoes and cold weather [3]. Migratory populations prevent against mosquito bites and cool weather by making fires. They use

cotton blankets because of their inability to afford bed nets [4].

Pyin Oo Lwin Township has been supplied with adequate rapid diagnostic test (RDT) kits and artemisinin-based combine therapy (ACT) at rural sub-health center level without interruption by vector borne disease control (VBDC) program since 2003. There has been also free provision of insecticide treated nets by non-government organization and international non-government organization for special activity sites since 2002. According to the yearly report by Pyin Oo Lwin Township, malaria morbidity and mortality rates become decreased along with these malaria control actions [5]. Although malaria prevalence was reduced, malaria was still endemic in the forested areas. This study was done to determine the socio-behavioral factors on malaria prevention and control among the forest

related workers in hard-to-reach areas of PyinOoLwin Township.

MATERIALS AND METHODS

Community-based cross-sectional survey method was carried out by collecting data using both quantitative and qualitative approaches. Study population consisted of people who were working at the forest related jobs such as, distant farming, fire-wood making, wood cutting, fishing, working at special activities like road and dam construction in the forested areas. The study was done at the forests, forested foot hill and special project sites located at hard-to-reach areas which are more than two hours walking distance away from health centers of Wat Won, Pyin Sar and Kyar Twin Yae village tracts. A total of 204 number of forest related workers were interviewed with pre-tested, closed ended questionnaire. Three focus group discussion (FGD) sessions were done for qualitative approach. Environmental risk factors were investigated using check-list to assess the factors relating vector breeding places in one mile distance from the houses of the respondents.

The responses in the questionnaires were compiled, coded, entered into computer using SPSS 11.5 software and analyzed by R 2.9.0 software. The associations between the determinants of malaria and disease occurrence were analyzed. The crude odds ratios (OR) with 95% confidence intervals (CI) were calculated. The education of the respondents was categorized as illiterate, primary, middle, high school, university and graduate level according to their levels at which they had attended. Education levels were given with marks from 0 to 5 according to their levels. The occupations of the respondents were also categorized as distant farmers, laborers, mechanic workers, job owners and government staff. In the same way, occupation levels were also given score from 1 to 5. Personal income of the respondents was also divided into three

groups with, income of less than 60000 kyats a month, 60001 to 100000 kyats a month and more than 100000 kyats a month. Similarly, income levels were given marks with score 1 to 3 according to their increasing amount of income.

According to the composite scale of the education, occupation and income of the respondents, the forest related workers were classified as skilled workers if the scale was 6 and above and classified as unskilled workers if the scale was less than 6. The disease occurrence was investigated using history of malaria in forest workers. Persons with history of malaria were defined as any workers who had history of confirmed malaria within two years according to diagnosis with RDT or microscopy at any health center or who had ever taken antimalarial treatment either for infection due to falciparum and vivax. Persons with no history of malaria were defined as workers who had no history of confirmed malaria according to diagnosis with RDT or microscopy at any health center and who had no history of taking antimalarial treatment within two years.

RESULTS

Characteristics of forest related workers

According to the interviews, age, sex, marital status, educational status, kinds of occupation in the forest and monthly income of studied population were described in Table 1. Among the workers, 88.7% of them worked in the day time. However, 11.3% worked their jobs both in the day and night time. About two thirds of the studied populations were working in the forest for two to three years. However, 38% of the workers had been working in the forest for longer duration, i.e. more than three years. More than half, i.e. 57% of the studied workers were working in the forested areas located within 8 miles distance from health centers. The rest were working in the areas located over 8 miles far from health centers. Among the forest related workers,

149 (73%) had history of malaria and only 55 (27%) were free from malaria in the last two years.

Table 1. Background characteristics of forest related workers (n=204)

Factors	Frequency	Percent
<i>Age (in completed years)</i>		
15 – 24	45	22.1
25 – 34	54	26.5
35 – 44	38	18.6
Above 44	67	32.8
<i>Gender</i>		
Male	130	63.7
Female	74	36.3
<i>Marital status</i>		
Unmarried	55	27
Married	149	73
<i>Education</i>		
Illiterate	19	9.3
Primary level	130	63.7
Middle level	36	17.6
High level	15	7.4
University level	3	1.5
Graduate	1	0.5
<i>Occupation</i>		
Distant farmer	160	78.3
Laborer, firewood maker, wood cutter	21	10.3
Mechanic	14	6.9
Job owner	5	2.5
Government servants	4	2
<i>Day/ night worker</i>		
Day time worker	181	88.7
Day & night time worker	23	11.3
<i>Monthly income</i>		
10000 kyat to 60000 kyat	164	80.4
60001 kyat to 100000 kyat	29	14.2
100001 kyat and above	11	5.4
<i>Working duration in forest</i>		
2 to 3 years	126	61.8
More than 3 years	78	38.2
<i>Distance between forest and health centers</i>		
8 miles and less	118	57.8
More than 8 miles	86	42.2
<i>Socio-economic status of the workers</i>		
Upper or skilled workers	62	30.4
Lower or unskilled workers	142	69.6
<i>History of malaria among the workers within two years</i>		
Yes	149	73
No	55	27

Knowledge on malaria

This study also assessed their knowledge on malaria concerning about cause of malaria, biting time of malaria vector, signs and

symptoms of malaria, diagnosis of malaria, treatment seeking places and sources of bed net impregnation. These results are shown in Table 2. Among the workers, 55.4% knew the cause of malaria (i.e. mosquito bite). However, 13.3% of forest workers misunderstood the cause of malaria and the rest did not give any comment.

Table 2. Knowledge on malaria (n=204)

Factors	Frequency	Percent
<i>Cause of malaria</i>		
Mosquito bite	113	55.4
Drinking stream water	19	9.4
Swimming or bathing in forest	8	3.9
Don't know	64	31.3
<i>Biting time of malaria mosquito</i>		
Night time	99	48.5
Day time	15	7.4
Don't know	90	44.1
<i>Knowledge on signs & symptoms of malaria</i>		
Know more than 3 symptoms	25	12.3
Know 1 to 3 symptoms	153	75
Don't know	26	12.7
<i>Knowledge on diagnosis of malaria</i>		
Rapid diagnostic test	30	14.8
Microscopy	122	57.4
Clinically	24	11.8
Don't know	41	20.1
<i>Knowing the treatment seeking places for malaria</i>		
Know the places (health center, clinic, hospital)	185	90.7
Don't know	19	9.3
<i>Knowing the persons who are malaria care providers</i>		
Know the providers (basic health staff etc.)	130	63.7
Don't know	74	36.3
<i>Knowing the sources of impregnating the bed net</i>		
Know the sources (when, where, who)	95	46.6
Don't know	109	53.4
<i>Knowledge on course of antimalarial treatment</i>		
Know the full course	99	48.5
Don't know	105	51.5

About half (48%) of the studied populations knew the biting time of mosquito causing malaria (i.e. night time). The correct time, place and person relating sources of impregnation of the bed nets in the community areas, were mentioned properly by about half of the studied populations (i.e. 46.6%). Half of the studied populations could mention about the full course of antimalarial treatment according to national guideline.

Practice and behaviors of forest related workers

A total of 204 forest related workers were interviewed to investigate their behavior and practice on personal protection against malaria. These are described in Table 3.

Table 3. Practice and behaviours of forest related workers

Factors	Frequency	Percent
<i>Sleeping with personal protection</i>		
Without any protection	102	50.0
With long sleeve	1	0.5
With repito application	1	0.5
Beside smoky fire	3	1.5
With blanket	34	16.7
With bed net	63	30.8
<i>Reasons for not using bed net (n=141)</i>		
Not habitual to	42	29.8
No mosquito and no bite	35	24.8
Not afford to buy	19	13.5
Hot	18	12.8
Not get enough air	15	10.6
No relevant reason	12	8.6
<i>Regular impregnation among bed net user (n = 63)</i>		
Impregnate the bed net regularly	6	9.5
Not impregnate the bed net	57	90.5
<i>Reasons for not impregnating the bed net (n = 57)</i>		
Not habitual to	2	3.5
No mosquito and no bite	1	1.8
Not afford to go	1	1.8
Not accessible	10	17.5
Not know sources (where, when, who)	35	61.4
No relevant reason	8	14
<i>Taking full course of drug among persons with malaria (n = 149)</i>		
No	29	19.5
Yes	120	80.5
<i>Reasons for not taking the full course of drug (n = 29)</i>		
Recovery from fever	11	37.9
Nausea, vomiting and dizziness	8	27.6
Kept the drug to take for the next malaria attack	4	13.8
Not afford to go	3	10.3
Change to another health provider	2	6.9
Not accessible	1	3.4
<i>Health seeking behavior</i>		
	1 st action n=149(%)	2 nd action n=55(%) 3 rd action n=15(%)
Self-medication	23(15.4)	2(3.6) 1(6.7)
Family member	4(2.7)	- -
Drug seller	4(2.7)	- -
Traditional healer	5(3.4)	1(1.8) -
Quack	6(4.0)	4(7.2)
Volunteer health worker	7(4.7)	3(5.4) 1(6.7)
Basic health staff	41(27.5)	22(40) 7(46.6)
Doctor	21(14.1)	17(30.9) 3(20.0)
Hospital	38(25.5)	6(10.9) 3(20.0)

Half of the studied workers did not sleep with any personal protection such as bed net, blanket, repito application, mosquito coils or sleeping beside smoky fire. Only 63 of them (31%) had behavior of sleeping with bed net in the forest. A total of 141 workers did not use the bed nets. The main reason for not using the bed nets was that they were not habitual to use the bed nets in the forest. Another foremost reason was that there was no mosquito in the forest. Third reason was that they were not affordable to buy the bed nets. Among the bed net users, only 9.5% impregnated their bed net regularly and 90.5% did not impregnate their bed nets regularly. The major reason for not impregnating the bed nets was that they did not know the time of impregnation, the place of impregnation and the persons who are conducting the impregnation of the bed nets in the community. Among 149 of the respondents who had history of malaria, 80% took the full course of anti-malarial treatments. The rests took partial treatments. The majority of them stopped the course as soon as they recovered from fever.

The second leading reason for not taking full course was because of fear of side effects of drug. According to health seeking pattern stated by the respondents, the majority of workers took the treatment from the health staffs at the first action. However, 15% of them took self-medication at home in the first action and some of them took self-medication at home even in the second and third actions for malaria episodes.

Environmental risk factors

According to the checklist of environmental risk factors for mosquito breeding places such as streams, rice fields, swamp, hoof-marks, cowshed and outdoor shelters were predominantly found in the 94%, 90%, 68%, 95%, 79% and 85% of the environments of residues of the respondents, respectively. Bamboo cottages and huts without wall were the commonest housing type resided by 50% and 47% of the forest related workers, respectively.

Bivariate and multivariate analysis with logistic regression

Forest related workers with the age of above 24 years, were 2.5 times more vulnerable to malaria infection compared to younger workers. Similarly, the workers who lived in the forest for more than 3 years were 2.2 times more susceptible to malaria infection than the workers who lived in the forest for shorter duration. The respondents who were living near the slow running streams were 4.9 times more likely to expose the infection than the others. In the same way, those living near the rice fields were 4.3 times more likely to expose to the infection than the others. The workers with the behaviors of sleeping without bed nets were 11 times more likely to get malaria infection than the bed net users.

Multivariate analysis with logistic regression was done with the variables which were significant in bivariate analysis. According to logistic regression, bed net non-users if they were old aged, living longer duration in the forest in presence of streams and rice fields near their houses were 13.5 times more liable to be transmitted by the infections than the others. These are explained in Table 4.

Focus group discussion

Three sessions of FGD were conducted separately at three villages: Wat Won, Pyin Sar and Kyar Twin Yae. Discussions were done with twenty males and ten females of workers with the age of 15 to 60 years. Both males and females were consisted in each group. Thematic ideas concerning signs and symptoms of malaria, cause of malaria and their practice and behaviors malaria prevention and control were discussed.

Theme concerning with signs and symptoms

The respondent from Kyar Twin Yae village said “*I had suffered from unconsciousness during malaria fever.*” The majority of respondents from each discussion said that “*We experienced with chilling, rigors and fevers during attack.*”

Table 4. Bivariate and multivariate analysis between risk factors and malaria occurrence

Bivariate analysis				
Factors	No malaria	Malaria	Crude OR (95%CI)	p value
Age				
15 to 24 years	19	26		
Above 24 years	36	123	2.5 (1.16-5.29)	0.013*
Duration of stay (In forest)				
2 to 3 years	41	85		
> 3 years	14	64	2.2 (1.06-4.75)	0.024*
Streams (In environment)				
Absence	8	5		
Presence	47	144	4.9 (1.33-19.83)	0.007*
Rice fields (In environment)				
Absence	12	9		
Presence	43	140	4.3 (1.55-12.42)	0.003*
Knowledge				
Biting time of mosquito				
Know	29	69		
Not know	26	80	1.3 (0.66-2.52)	0.4
Behavior				
Sleep pattern				
Sleep early	5	10		
Sleep lately	50	139	1.4 (0.35-4.72)	0.5
Bed net using				
User	38	25		
Non-user	17	124	11 (5.12-24.2)	0.000*
Multivariate analysis with logistic regression				
Factors	Crude OR (95%CI)		Adjusted OR (95%CI)	P (LR test)
Bed net				
Non-user vs user	11.1 (5.42-22.67)		13.5 (6.06-30.2)	<0.001***
Duration of stay				
>3 years vs 2 to 3 years	2.2 (1.11-4.39)		2.6 (1.11-6.09)	0.023*
Running streams				
Presence vs absence	6.2 (1.78-21.42)		5.5 (1.13-26.94)	0.032*
Rice fields				
Presence vs absence	4.3 (1.71-11)		3.0 (0.91-9.62)	0.073
Age				
Above 24 years vs 15 to 24 years	2.5 (1.24-5.02)		1.9 (0.79-4.58)	0.156

LR test = Logistic regression test

These discussions were practically same as the symptoms of malaria disease.

Theme relating with causes of malaria

The participants from Pyin Sar and Kyar Twin Yae villages talked about the causes of malaria with *“We got malaria when we had unsafe water and fruit in the forest.”*

However, most of the participants of three groups discussed similarly as those answered in the questionnaire with *“Mosquito bite might make malaria.”*

Some participants from Wat Won village also said that *“We suffered malaria if we had imbalanced body temperature and reduced resistance to disease.”*

Strangely, one participant from Wat Won village shared his experience with *“Surely, one would get malaria if he did taboo or wrong doing in the forest.”*

Theme relating practice and behavior

Some of the participants from Pyin Sar and Kyar Twin Yae villages talked about self-medication as responded in questionnaire with *“Malaria was cleared if we took self-medication orally with combination of dried viscera of porcupine, carbon of fire smoke and turmeric powder.”*

Majority of participants of three groups pointed out their practices with *“We were secured from malaria by drinking safe or boiled water, sleeping beside fire and eating safe food.”*

Some discussed as suitably as they answered in the questionnaire *“Sleeping under the bed net was good if we could do like that.”*

Unexpectedly, some participants from Kyar Twin Yae said their reason for not using bed net with *“Oh! hanging of bed net was inconvenient for sleeping sites in the forests.”*

Some participants of Wat Won also gave reason with *“We had no extra bed net to use in the forest. We possessed only one which was just enough for our family.”*

This discussion was the same as the responses in questionnaire.

DISCUSSION

Majority of people in this study area expressed the inadequate understanding of malaria transmission and symptoms. On the contrary, a study in Indonesia found that 69% of people correctly responded with mosquito bite as causes of malaria. Incorrect responses included stagnant water, bad climate, unclean environment and unsafe food in that study [6]. Regarding symptoms of malaria, majority of respondents from both studies could state the correct symptoms. The present study found that only 55.4% of respondents had knowledge on cause of malaria with mosquito bite, 9.4% with drinking stream water and 3.9% with bathing or swimming in the forest.

Majority of the people in this study area had proper health care seeking practice for malaria. They took health care from health staffs of proper health centers with full course of antimalarial treatment. Only 20% of them did not take full course of treatment as they had misunderstanding in treatment completion course. Study in northern Sri Lanka found that the main reasons for not taking full course of treatment were side effects (57.6%) and clear of symptoms (16.7%) [7]. Similarly, the present study found that the reasons for not taking full course regime, were given by 37.9% and 27.6% of respondents with recovery from symptoms and fear of side effects, respectively. Survey of treatment seeking behavior in rural Ethiopia showed that community health workers were the source of the first action treatment seeking persons [8]. The majority of respondents in the present study took health care from basic health staff in every action for malaria episodes. However, fifteen percent of participants took self-medication in the first action. Therefore, most of people in this area had good health seeking behaviors although some took self-medication at home.

Risk factor survey in Thailand also detected that population movements, irregular use or non-use of mosquito nets, behavioral risk

factor such as delayed treatment, improper treatment seeking pattern were main risk factors for malaria infection [9]. Similarly, some risk factors for malaria appeared from this study. Older age, living longer duration in the forest, non-utilization of bed net and presence of environmental factors such as running stream and rice fields were significantly associated with disease occurrence in the present study.

Risk factors survey in the Philippines revealed that age greater than 30 and being a migrant were all found to be significant risk factors [10]. In the same way, the present study also found that the age older than 24 years and being a migrant were significant risk factors.

Conclusion

The forest workers in the study area had inadequate knowledge on malaria. The majority of respondents had very risky behavior like non-utilization of bed net in the forest. Their reasons for not using bed nets were unaffordable condition and inconvenience for hanging of bed nets in the forest. This study also revealed another risk behaviors of non-impregnation of bed net regularly although there were availabilities of impregnation. Moreover, they had behavior of taking self-medication with traditional plants and animals' viscera. According to this study, bed net non-users if they were old aged, living longer duration in the forest in the presence of streams and rice fields near their house, had greater risks for getting malaria infection than the others.

Recommendations

- (1) The forest related workers residing in the forested area should be educated with health issues concerning malaria prevention and control.
- (2) The forest related workers should be encouraged the practising the utilization of insecticide treated net and regular impregnation.
- (3) The forest related workers should be supplied with specific kind of bed nets

those are convenient for using in the forested sites.

- (4) They also should be given information on awareness of accurate sources of impregnation of bed net in the community.

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**Success and challenges of Public-Private Mix DOTS initiatives in Myanmar:
a process evaluation for partnership approach of non-governmental organizations**

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The study was conducted in three townships in which Public-Private Mix DOTS (PPM-DOTS) was implemented by three partners - Myanmar Medical Association (MMA), Population Services International (PSI) and Japanese International Cooperation Agency (JICA). It aimed to describe the process of implementing PPM-DOTS by the partners and to elicit their opinions on PPM-DOTS in order to take necessary actions. This study was a process evaluation which included desk review, opinion survey, in-depth interviews, key informant interviews and participation and observation of advocacy meeting and training on PPM-DOTS. Initiations of PPM-DOTS by each partner were different although all coordinated with National Tuberculosis Programme (NTP). More than half (54.3%) of general practitioners (GPs) had moderate level and 34.3% had very high recognition and acceptance of the need for partnership. For development of clarity and realism of purpose for partnership, 54.3% had high score. Most GPs convinced that their previous practices were not in line with NTP guidelines. Majority pointed out that proper advocacy for PPM-DOTS was crucial for success of future activities. Most respondents were aware many GPs needed to be involved, especially, GPs who were very good general practitioners known as "GP Kings". Almost all respondents highlighted that if there are fewer burdens for paperwork, more GPs would get involved in PPM-DOTS. Majority mentioned existing good personal relationship as success factor for PPM-DOTS. They also expressed trust building could be obtained by sharing information through regular contacts among each other. Based on the research findings, coordination mechanism among the partners in two pilot townships is initiated.

INTRODUCTION

There are many circumstances that are favorable for the initiation of Public-Private Mix Directly Observed Treatment Short course Strategy (PPM-DOTS) in Myanmar: political and economic changes, the introduction of an open market economy, increased demand by the general public for quality services, limited resources in the public sector, an increased proportion of public staff working also in the private sector, the increased involvement of non-governmental organizations (NGOs) in health care services, urbanization and

diversity of health care providers in peri-urban and slum areas, and sub-standard practices in private sector. Most TB patients (73.3%) at public tuberculosis (TB) centre first sought care at general practitioners' (GPs) clinics [1]. Many patients (38.8%) shopped around more than two GP clinics before seeking care at public sector [1]. However, the current practices of GPs were not in line with National Tuberculosis Programme (NTP) guidelines [2, 3]. The rate of multi-drug resistant TB (MDR-TB) was significantly higher in patients sought care at GP clinics first before seeking care at public sector [4]. All these circumstances

were interrelated leading to the introduction of the public-private partnership. The incorporation of the private sector into the public sector to deliver DOTS in different ways could meet the community needs, shorten the diagnostic delay and prevent emergence of MDR-TB.

PPM-DOTS focusing on involvement of private GPs in TB control in Myanmar has been established since 2003. Currently, there are three NGOs involved in PPM-DOTS: Population Services International (PSI), Myanmar Medical Association (MMA) and Japanese International Co-operation Agency (JICA).

The MMA and JICA mainly aim for Scheme I for all GPs which stipulates that GPs refer chest symptomatic patients to the public sector and that they provide health education messages to TB patients. Scheme II includes all components of Scheme I and stipulates GPs provide Directly Observed Treatment (DOT) to their patients. The PSI implements Scheme III of PPM-DOTS which includes the provisions of all items specified in Scheme II and mandates GPs to run their clinics as NTP approved centres, i.e. diagnose and categorize TB patients, keep records and report TB cases to the NTP.

Although there are few success stories of PPM-DOTS in Myanmar [5], there is no study which searches for explanation of success and challenges. A process evaluation focuses on the internal dynamics and actual operations of a programme that attempts to understand its strengths and weaknesses [6].

In contrast to outcome evaluation which assesses impact of programme, process evaluation verifies what the programme is and how it is implemented. It also aims to provide managers feedback on the quality of implementation and increase utilization of research findings. For future planning and effective implementation of PPM-DOTS, it is necessary to assess the process of activities by different partners and to utilize

lessons learnt from pilot projects in scaling up of PPM-DOTS in Myanmar.

Objectives

- To describe the process of implementing PPM-DOTS in Myanmar by MMA, JICA and PSI in collaboration with NTP
- To elicit lessons learnt and opinions of key partners on PPM-DOTS in order to take necessary actions
- To enhance utilization of research findings for more effective expansion of PPM-DOTS

MATERIALS AND METHODS

Study design

It was a process evaluation including both qualitative and quantitative methods.

Study areas

One township for each partner regardless of implementing schemes was selected. Three townships - Pyae, Pyin Oo Lwin and North Okkalapa Townships - were selected after discussing with three NGOs for PPM-DOTS and NTP.

Data collection

Desk review

All available documents on PPM-DOTS including policy guidelines, NTP annual reports, WHO review mission reports, conference proceeding or presentations and meeting minutes were reviewed and analyzed.

Opinion survey

It was conducted with all GPs (n=35) involved in PPM-DOTS with MMA, JICA and PSI by using pre-tested self-administered structured questionnaire including 10 questions. It was based on two principles of partnership: recognize and accept the need for partnership, and develop clarity and reasons of purpose for partnership [7]. The responses were measured in 4 point Likert scale -- strongly agree, agree, disagree and strongly disagree.

In-depth interviews (IDIs)

They were conducted with 13 GPs.

Key informant interviews (KII)

A total of 8 KIIs were conducted with public staff, Township Medical Officers (TMOs), Township TB Coordinators (TCs), Franchising Officer (FO) and Out Reach Workers (ORWs) from study areas to find out their perceptions towards partnership and collaboration.

Observation

Investigators participated and observed key activities on PPM-DOTS by different partners such as advocacy meetings, trainings and evaluation meetings.

Data analysis

Quantitative data from the opinion survey were cleaned and entered in SPSS version 11. Descriptive analysis was carried out by calculating frequencies and percentages. The scoring was given for responses to questions related to two principles of partnership. For the principle A (recognize and accept the need for partnership) and principle B (develop clarity and realism of purpose for partnership), scores were given as 16-20=high, 11-15=medium, 6-11= low and 5=very low. For aggregated score, it was given as 31-40=high, 21-30=medium, 11-20 = low and 10=very low, respectively [7].

Qualitative data were organized with the assistance of ATLAS ti version 5.0 software to conduct code retrieval, create cross link between different transcripts and networking among relevant code family. Systematic contrasting and comparing among cases was carried out by sorting out similarities and differences.

Ethical consideration

Researchers followed the principles and guidelines of ethical consideration for privacy and confidentiality. This study obtained ethical approval from Ethical Review Committee of Department of Medical Research (Lower Myanmar).

RESULTS

Initiation of PPM-DOTS

Initiation of PPM-DOTS included how each partner conducted advocacy meetings, trainings and organizing GPs. The approach to initiate PPM-DOTS by each partner was slightly different although all coordinated with NTP. The PSI organized GPs through existing franchising network, MMA and JICA conducted advocacy meeting at township and organized GPs through existing continuing medical education (CME).

Advocacy meeting and training

For PSI, GPs were trained in batches, via a three-day training course for Scheme III. Once trained, the GPs received branded DOTS signboards to identify their clinics as sites for free TB treatment and diagnosis. The NTP provided TB drugs free of charge and PSI repacked in branded boxes and distributed to the Sun Quality Health (SQH) doctors. The JICA conducted advocacy meeting first before the training, TC went to GP clinics and invited the GPs for advocacy meeting. The MMA conducted advocacy meeting together with training of GPs. Personnel from NTP provided technical support and guidance for the training. The MMA implemented all schemes according to GP's preferences. Findings from KII and field observation showed only selected GPs were invited for advocacy meeting. Thus, not all the GPs in the township were informed about PPM-DOTS.

How GPs involved in PPM-DOTS

For PSI, SQH-GPs involved in PPM-DOTS through existing franchising network. Most GPs who involve in reproductive health, malaria and sexually transmitted infection projects were invited to attend training on DOTS. For JICA and MMA, GPs involve in PPM-DOTS through existing Continue Medical Education (CME) programme. GPs who attended regular CME were invited to attend PPM-DOTS training. At that time, they were not well informed about PPM-DOTS activities and their responsibilities.

Majority of respondents pointed out that initiation and advocacy for PPM-DOTS was crucial for success of future activities. It was also essential to desensitize public staff, physicians and GPs for essence of partnership, sharing information and credits among each other.

Understanding the concepts of PPM- DOTS

Some GPs perceived that the reason for initiation of PPM-DOTS might be due to the unsatisfactory case detection rate by public sector. Almost all GPs stated most patients seek their health care in private clinics and only by means of PPM, TB patients in the private clinic can be detected and be able to provide the appropriate treatment.

"The first contact of the patients is GP and all kinds of patients become intimate with us and they discuss with us in detail. They reveal their feelings frankly. So we can detect TB cases and persuade them to examine sputum etc. GPs play a key role in case detection".

(GP, 10 years GP service, implements Scheme I)

Partnership principles

More than half of GPs (54.3%) had medium level score and 34.3% of GPs had very high recognition and acceptance of the need for partnership, respectively. About 54.3% had very clear and realistic purpose of partnership (Fig.1).

Roles and responsibilities

The MMA-TC, JICA-TC and FO had different tasks, background and responsibilities according to implementing schemes and data needed for individual projects and funding agencies although all were local persons for PPM-DOTS in their respective townships. At advocacy meeting and trainings, GPs were informed about their responsibilities in general such as to refer TB suspects. Majority of GPs did not expect to take care of recording although they were aware to provide other tasks like providing health education.

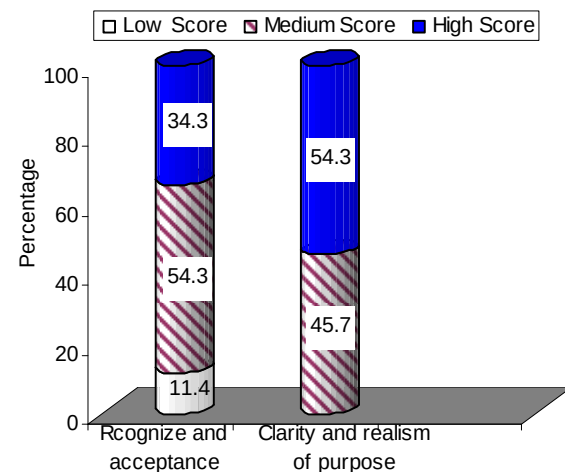


Fig.1. Opinions of GPs on partnership principles

"At first, I knew there were three schemes. Since I chose Scheme I, I need to refer TB suspects. I also explain patients to examine sputum and how to collect sputum. Then TB department examined sputum, diagnosed and provided treatment. They gave feedback to us."

(GP, 4 years GP service, implements Scheme I)

"Our responsibility is to detect TB cases and treat them. Others will be taken care by FO such as recording, reporting and defaulter tracing."

(PSI- GP)

Lessons learnt

Strengths

Main strength of PPM-DOTS mentioned by most respondents was benefits for TB patients. They can get treatment free at GP clinics which was more convenient for them. Increasing case detection, gaining new knowledge and better case management skill for GPs were also regarded as opportunities for GPs. Only a few expressed having risk of contracting TB and increasing workload especially paperwork as threads of PPM-DOTS.

"We are very happy to involve in PPM-DOTS especially as it has benefit for patients. That is the main strength. Weakness or risk is getting chance of TB infection. But as you know, we're exposed to

all kinds of infections. So there is no difference."

(GP, 24 years GP service, implements Scheme III)

"Some GPs are reluctant to implement Scheme III especially whose clinics are attached to their home. They concerned of contracting TB infection to their family especially children."

(MMA Staff-1)

Challenges

Challenges in implementing PPM-DOTS as identified by respondents were as follows:

- Burden for recording
- Frequent change of recording formats
- Reluctance of specialists to refer their cases
- Disorganizing in using anti-TB drugs/patient kits by some GPs
- Wrong address given by some patients
- Difficulty in establishing private lab for Scheme III
- Some delay in issuing anti-TB drugs

Almost all respondents (GPs and key informants) highlighted problems in less familiarity to recording thus required to keep minimum and essential facts. Formats for records were not standardized and changed frequently. Some stated specialists did not want to refer their cases because they were afraid that medical officer would criticize their diagnosis. So specialists referred only confirmed sputum-positive cases to NTP and kept sputum-negative cases at their clinics. Although FO and ORW assisted PSI-GPs for recordings, they mentioned that few GPs were not well organized in using patient kits. Delay in issuing anti-TB drugs from NTP had been solved by providing patient kits during the study period.

Although many GPs were reluctant and complained for recording and implementing Scheme III, one PSI-GP shared her experience as a good example to make it simple:

"Many people asked me how I could manage so many cases. I can take care twice of existing patients, I would say. Patients find their kits by themselves. I've shown them how to do that at first time. Patient' kit has number (Fig.2) and so he/she can find it and put it on this (showing a desk). Then I come out from examination room and give drugs and mark tick on treatment card. I only need to record treatment card. Others are taken care by FO. "

(PSI- GP)

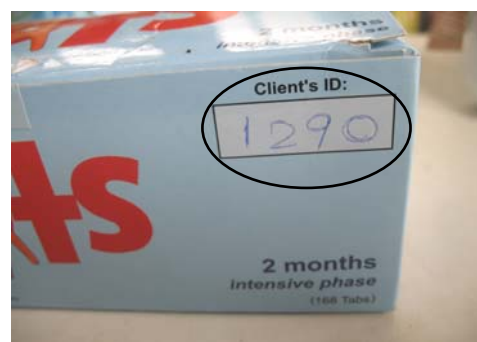


Fig. 2. TB patient kits showing patient's ID

Success

Indicators for success

Success indicators for PPM-DOTS as expressed by respondents were as follows:

- Increased number of cases/case load
- Improved case holding
- Decreased number of defaulters
- Increased number of TB cases detected
- Increased number of cure patients
- Increased number of GPs attended advocacy meetings and trainings

Contributing factors towards success

Almost all respondents pointed out good personal relationship as one of the key factors for success. Most GPs and key informants expressed understanding and trust building could be obtained by sharing information and regular contacts among each other. They pointed out that all should bear in their mind that they were working for TB patients and not for their personal

benefits or credits. Existing CME was a good way to organize GPs. Some GPs highlighted that uninterrupted supply of anti-TB drugs, enthusiasm and interest of TCs were also crucial for success and sustainability of PPM-DOTS activities.

"For success of PPM-DOTS, uninterrupted drug supply is essential. Understanding among each other and coordination are also important. We all should aware that we're working together for TB patients. It's not for our individual benefits. If we all convinced that, we can negotiate, understand, forgive even though there are some misunderstandings, conflicts or problems."

(GP, 15 years GP service, implemented Scheme II)

Suggestions

How to organize GPs

Proper advocacy and training played a key role to organize more GPs. Majority of respondents stated that GPs would be interested if they could learn recent treatment guidelines. Some also suggested meeting GPs in person by going from one clinic to another instead of sending invitation letters to them.

"I think it's better to go from one GP clinic to another and explain them well. It's more effective than just sending invitation letters to clinics. If we cannot persuade them once, we better try twice, thrice ect."

(GP, 30 years GP service, implemented Scheme III)

"Most GPs would come if they are invited..... and if there is no pressure for them. Pressure means... you know, if they think they are forced to do or something like that. GPs are independent people and so organizing GPs is different and delicate. Invitation letter should include agenda. GPs will come if they think they can learn something."

(GP, 12 years GP service, implemented Scheme III)

Some respondents pointed out that PPM should be targeted to the GPs who are permanently residing in local area and have many patients known as "GP Kings".

If I have to do this (organizing GPs for PPM-DOTS), I will make a list of "GP Kings" in this township, first. Then I will go to them and discuss in person for their contribution. There should be recognition or acknowledgement or incentives for them as well. If they do not come to us, we will have to go and meet them. I think it will work.

(GP, 12 years GP service, implements Scheme I)

Keep recording minimum and simple

Almost all key informants and GPs highlighted there should be fewer burdens of paperwork. They all convinced that if there were less paperwork, more GPs will involve.

"GPs did not expect to do such paperwork when they start involve in PPM-DOTS. We haven't told about that much. Now it becomes huge burden for GPs. So, if you ask me, I would say to reduce paperwork."

(MMA Staff-1)

"I want the PPM project to reduce paperwork and recording and keep them simple and minimum. Now, it becomes more and more data needed. If it happens like that, I'm afraid most GP would run away. GPs want to be independent and not to take care about data or records. They are only interested in clinical issue and treating patients. If there is more paperwork, PPM will not be successful and sustainable. Please consider this point seriously to expand PPM-DOTS for the whole country. Kindly include this fact in the report."

(NTP Staff-2)

Enablers

Almost all respondents pointed out that PPM-DOTS signboards of GPs' clinics should be striking so that general public would notice and impressed. Majority of key informants mentioned that appreciation

of individual GPs was also important since they did not have any financial incentives for involving PPM-DOTS. On the other hand, a few highlighted that it was essential to ensure incentives as motivating or enabling factors but not creating problems among implementing GPs and partners. Some emphasized that enablers or incentives for GPs and for patients provided by different partners should be similar.

"If budget permits, there should be better or grand arrangement for gathering of GPs - may be once or twice a year at the famous restaurant to exchange their experiences. And prominent or better quality signboard for MMA PPM-DOTS should be provided...Now it is not so impressive."

(Public Staff-3)

Sustainability

Regular drug supply, regular meetings, monitoring and supervision, recognition or acknowledgement of GPs for their contributions and motivation in kind of informal meeting were suggested by most of the respondents for sustainability of PPM-DOTS. One public staff suggested to send timely reminder to GPs who do not refer TB suspects and to acknowledge GPs who has detected many cases:

"For GPs, there should be regular reminder or acknowledgement about their referral. So they would become aware and interested in PPM DOTS. If such letters come from higher level, or divisional level, it's better."

(Public Staff)

DISCUSSION

Initiation and advocacy for PPM-DOTS was crucial for success of future activities. It was also essential to desensitize public staff, physicians and GPs for essence of partnership, sharing information and credits among each other. At the beginning of PPM-DOTS, GPs were not well informed about its activities and their responsibilities. The study conducted in 2003 found that 67.4%

of GPs have heard about PPM [8]. In our study, most GPs had concept of PPM and positive aspects towards PPM-DOTS in terms of gaining new knowledge and convincing their previous managements were not in line with NTP guidelines.

More than half of GPs (54.3%) have moderate level and 34.3% had very high recognition and acceptance of the need for partnership, respectively. Acceptances of GPs for basic principles of partnership were good. The most concern for GPs as well as key factor for success of PPM-DOTS was found as less paperwork. Recording format was not standardized though all partners followed NTP guidelines. Most respondents suggested keeping recording minimum and simple which was similar to findings from previous studies [3, 8].

The study in two peri-urban townships in Yangon showed that lack of trust between public and private sector as barrier for partnership [8]. In our study, there was certain level of trust and understanding between public and private sector. However, most respondents also expressed weak coordination among PPM partners at the township level and limited awareness of each other's activities. This finding was similar to the findings from previous study that township level coordination was essential for successful partnership [8]. Trust building among all partners, public staff and GPs should be enhanced further by sharing information and regular contacts.

Conclusion

Good personal relationships among each other, fewer burden for paperwork, regular CME programme, regular drug supply, regular contacts and coordination among partners were main factors for successful and sustainable PPM-DOTS based on the research findings, coordination mechanism among the partners in two pilot townships has been initiated. The MMA aims to engage all GPs in PPM-DOTS by inviting all GPs for advocacy and to develop new signboard for MMA PPM-DOTS and to

keep paperwork and recording simple and minimum.

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SHORT REPORT

Self-medication practices in a peri-urban area of Yangon City: a qualitative approach

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Drugs or medicine can be generally classified into prescription drugs (Rx) and over-the-counter (OTC) drugs. Prescription drugs mean the drugs those can be consumed only by doctors' prescription. The drugs those can be consumed without doctors' prescription and those can be bought freely from drug stores are known as OTC drugs [1]. The practice of self-medication has been a feature of healthcare for many years. Although OTC drugs are the commonest form of self-medication, a person may also self-medicate by taking more or less than the recommended dose of a prescribed drug [2]. Poor socio-economic status, high cost of modern medicine and non-availability of doctors in rural areas lead to difficulties in accessing modern healthcare. Drug retail shops frequently serve as the public's first point of contact in health care system. The practice of indiscriminate self-medication is a long lasting problem in Myanmar Health Care System.

This study was conducted with the general objective of obtaining information about groups' beliefs and self-medication practices among community residing in a peri-urban area of Yangon City. The study used focus group discussion (FGD) being carried out in low socio-economic community in Number 5 Ward of Hlaingthaya Township of Yangon City from July to October, 2009. Focus group discussions with 7 people in each FGD were done following an informed consent. Four FGD sessions emerged: female group 18-35 years old, male group

18-35 years old, female group 36-60 years old, and male group 36-60 years old.

Almost all of the participants in older age group of both sexes responded that they were the persons who made decisions for taking treatment not only for themselves but also for family members and other household persons if they got some kind of illness. Participants of younger age group said that heads of the family or elders usually gave decisions for treatment and the individual who suffered illness usually followed this instruction.

Among self-medication, buying drug package from drug store was the commonest. The reason was that it was the most convenient way for them in terms of availability, accessibility and affordability. They also considered that health knowledge of pharmacy staff (from drug stores) was much better than themselves. They were familiar to some drugs frequently advertised in magazines, journals and television, and usually took these drugs first if they got sick.

Going to clinic was the last option according to the participants interviewed. They did not like the repeated appointments of the clinics and they thought that it was a way of doctors to take money from their patients. Not only the participants took drug packages sold by drug stores, but also they relied on *say-mee-to* (ဆေးမြီးတို့) (herbal medicine or folk medicine). A variety of *say-mee-to* regimes were used to treat their own illnesses.

The worst scenario expressed by some participants was consultation with quacks to treat their illnesses. They not only gave tablets, but also did injections and even minor surgeries.

One of the factors influencing self-medication was high health care cost. Some interviewees argued that establishment of free-of-charge clinics run by international NGOs or well wishers could reduce self-medication practices. Even if resources are adequate, every free-of-charge clinic in health care can reduce free-rider and other unwanted problems among community.

However, financial status of the community is the single most important factor for decision of treatment. Since this study was conducted among the community with very low socio-economic status, almost every participant confessed that they self-medicated at least once in their lifetime just

because they did not have enough money to go to the clinics. Further community-based studies are required on factors influencing self-medication and non-doctor prescribing and community surveys to delineate the folk-professional boundary of medication practices. Public education should be focused on proper medication practices and danger of treatments with unlicensed practitioners/injectionists.

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INDEX TO VOLUME 21 (1-3), 2009

AUTHOR INDEX

A

Aung Thu	74
Aye Aye Myint	62, 152
Aye Aye Than	93
Aye Aye Win	1
Aye Aye Yee	83
Aye Hnin Thwe	178
Aye Maung Han	104
Aye Myint Oo	22
Aye Than	17, 26
Aye Thaung	104
Aye Yin Shwe	44

B

Bo Myint	98, 186
----------	---------

C

Cho Cho	164
Cho Cho Myint	186
Cho Cho Win	164

H

Han Win	38
Hay Mar Hpoo	1
Hla Phyo Lin	17
Hla Soe Tint	88, 98, 117, 178
Hnin Lwin Htun	22
Hnin Nwe Oo	44
Htein Lin	194
Htin Zaw Soe	65
Hyunah Kim	56, 126

K

Kay Thi Aye	32
Khaing Win Htun	7

Khin Chit	132
Khin Htet Zin	178
Khin Htwe	32
Khin Khin Aye	74
Khin Lin	178
Khin May Nyo	17
Khin May Ohn	144
Khin May Oo	83
Khin May Thi	38
Khin Myat Tun	38, 93, 104, 132
Khin Myo Aye	164
Khin Nwe Oo	7, 121
Khin Phyu Pyar	78
Khin Pyone Kyi	56, 83, 126
Khin Sandar Oo	186
Khin Tar Yar Myint	17, 93, 132
Khin Thet Wai	121
Khine Khine Lwin	17, 93
Khine San Yin	62
Khine Win	83
Khine Zar Win	121
Ko Ko Zaw	50, 119
Kyaw Hlaing	194
Kyaw Kyaw	117, 121
Kyaw Myint	121
Kyaw Oo	119
Kyaw Zin Thant	32, 117
Kyi Kyi Thinn	110
Kyin Hla Aye	164
Kyu Kyu Than	50, 171

L

Lwin Lwin Maw	1
Lwin Zar Maw	22

M

Mar Mar Myint	17
Marlar Aung	178
Marlar Than	78
Maung Maung Mya	157
May Aye Than	12, 17, 26
May Thu Kyaw	22
Mg Mg Htay	12
Mg Mg Myint	1
Mi Mi Aye	12
Mi Mi Htwe	121
Mie Mie Nwe	22
Min Min Win	62
Moe Kyaw Myint	178
Moe Thida Kyaw	104
Moe Wint Oo	26
Moh Moh Hlaing	104
Mu Mu Sein Myint	17
Mya Mya Aye	44
Mya Mya Win	1
Mya Thet Lwin	26
Myat Phone Kyaw	78, 164
Myat Thida	44
Myint Kyi Aye	1
Myint Myint Hla	62
Myitzu Tin Oung	88, 98, 117, 178
Myo Khin	83
Myo Myo Mon	119, 138
Myo Win	152
Myo Zaw	38

N

Nilar Win	152
-----------	-----

O

Ohmar Lwin	83
------------	----

P

Pe Than Htun	65, 157
--------------	---------

Pe Thet Khin	32
Phyu Phyu Aung	93, 104
Phyu Phyu Win	17
Pyone Thuzar Nge	138

S

San Aye Kyi	152
San San Aye	138
San San Oo	83
Sandar Lin	32
Sandar Kyi	38
Sandar Nyunt	74
Saw Lwin	65
Saw Saw	119, 138, 171, 186
Sein Min	65, 157
Sein Thaug	65, 157
Soe Min Thein	1
Soe Thein	65
Suk-Hoon Ha	56, 126

T

Than Nu Shwe	104
Than Soe	12
Thandar Lwin	119, 186
Thandar Min	186
Thandar Myint	132
Thar Tun Kyaw	88
Thaug Hlaing	65
Thaw Zin	78, 132
The Maung Maung	50
Thein Tut	26
Thein Thein Myint	104
Thein Tun	88
Theingi Myint	138
Theingi Thwin	22, 93, 104
Thet Min Tun	194
Thet Thet Mar	22
Thi Thi Khaing	178

Thida	117, 186
Thidar Htwe Win	117
Thidar Khine	93
Thindar Maw	32
Tin Htun Hla	26
Tin Oo	164
Tin Ko Ko Oo	22
Tin Mi Mi Khaing	186
Tin Myint	32
Tin Tin Wynn	117
Tin Tun	144
W	
W. Tun Lin	65
Wah Wah Aung	44, 110
Wai Wai Myint	138
Win Aung	56, 74, 126

Win Win Maw	12, 132
Win Win Myint	78
Y	
Yae Chan	38
Ye Tint Lwin	1
Yee Yee Lwin	32
Yeon Hee Kim	56, 126
Yi Lwin Kyaw Zaw	44
Yi Yi Kyaw	83
Yin Thet Nu Oo	50, 119, 138
Yin Yin Khaing	32
Z	
Zaw Myint	1, 22
Zaw Win Tun	117
Zayar Htun Tin	171

SUBJECT INDEX

A

ABO blood-group system	74
Acquired immunodeficiency syndrome	171
Adolescence	50, 138
Adult	38, 78
Anopheles	157
Antibodies, viral	32
Anti-inflammatory agents	26
Antimalarials	78
Antioxidants	12, 132
Antitubercular agents	98, 186
Ascorbic acid	152

B

Beriberi	104
Blood donors	74
Blood glucose	22
Body constitution	1

C

Cardiovascular system	1
Chemoprevention	7
Cloning, organism	56, 126
Cost of illness	119
Culture	117
Cytogenetics	157

D

Dengue	32, 65
Diabetes mellitus	22
DOTS	186

E

Enzyme-linked immunosorbent assay	121
<i>Escherichia coli</i>	56
Fishes	144

F

Food contamination	93
Food preservation	44

G

Gingivitis	26
------------	----

H

Hepatitis B surface antigens	56, 74, 83, 126
HIV	171
Hypoglycemic agents	17

I

Insect vectors	65
----------------	----

K

Knowledge, attitudes, practice	117, 171
--------------------------------	----------

L

Lactate dehydrogenase	164
Lead-toxicity	93
Learning	144
Leprosy	7, 121
Ligation	126

M

Malaria	88, 157, 164, 178
Malaria, falciparum	78
Malondialdehyde	152
Medical staff	88, 138
Membrane proteins	121
Milk, human	104
Mouthwashes	26
Myelodysplastic syndromes	62

N

<i>Neisseria gonorrhoeae</i>	110
------------------------------	-----

O

Onions 22

P

Parents 50

Plant extracts 12, 26

Plants, medicinal 17, 26

Plasmids 56, 126

Preeclampsia 152

Pregnancy complications 152

Private sector 186

Public sector 186

R

Reproductive medicine 50, 138

Risk factors 1, 38

Rural population 88, 117

S

Sexually transmitted diseases 110

Shrimp 44

Smoking 38

Social behavior 178

Students, nursing 144

T

Tea 12, 132

Thiamine 104

Thorax 117

Tuberculosis 98, 119, 186

Tuberculosis, pulmonary 38

V

Vitamin E 152

Y

Yeasts 126