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Quality assessment on care of Sexually Transmitted Infections in AIDS-STD clinics at the district level

Soe Myint, Aye Moe Moe Lwin & Kyawt Sann Lwin

Department of Health Planning

Quality assurance of care on Sexually Transmitted Infections (STI) is an important measure to control the spread of HIV/AIDS in Myanmar. In order to improve it, quality assessment of STI case management at the AIDS-STD clinics was carried out. The study employed a cross-sectional design with observation on patient-provider encounters, interviewing of health staff and patients, reviewing records and registers from seven AIDS-STD clinics for measuring STI service indicators 1, 2 and 3. Myanmar National Standard Treatment Guidelines for Management of STI was used as standard for treatment of STI cases. The study reveals that achievement for STI Service Indicator 1 is 2.1%, for STI Service Indicator 2 is 8.5% and for STI Service Indicator 3 is 0% out of seven AIDS-STD clinics. The study recommends that STI diagnosis and treatment, counseling and advice, and essential STI drug supply should be improved by proper training, supervision and management.

INTRODUCTION

An estimated 340 million new cases of largely treatable sexually transmitted bacterial infections occur annually around the world and more than 100 million new cases are young people aged 15 to 24 years [1]. Many of them remain untreated because they are either unable to diagnose or lack competent and affordable treatment services. In addition, millions of cases of incurable viral infections occur annually including five million new HIV infections, half of which affecting young people and 600,000 affecting infants owing to mother to child transmission. Sexually Transmitted Infection is 14th priority disease designated in National Health Plan for introducing prevention and control measures in Myanmar because 150,000 cases of STIs including 23 % of Commercial Sex Workers (CSWs) and 35 % of Men Sex with Men (MSM) were estimated to be treated in the country [2]. As there is a huge caseload for STIs, National AIDS Program (NAP) is

taking responsibility for prevention and control of HIV/AIDS and Sexually Transmitted Infections (STI) since 1990. The fundamental goal of the HIV/AIDS and STI control program is early detection and treatment of the disease, preferably at the point of the patient's first contact with the health system. Currently, forty-five AIDS/ STD prevention and control teams are able to achieve this goal throughout the country because staff is well trained and teams are strategically located in all States and Divisions of Myanmar [3]. In STI prevention and care, one important task is adequate case management of patients with STI. This includes diagnosis, treatment, individual health education, counseling and partner notification [4]. Therefore, National Standard Treatment Guidelines for Management of STIs was developed and health workers were trained according to the guidelines [5].

Since the mid-1980, there have been a number of external quality assessments that examine the degree to which care in

developing country programs corresponds to program standards. The studies emphasized on direct observation of service delivery process usually supplemented by interviews and inventory of resources. With remarkable consistency, these studies have documented large, widespread discrepancies between care programs planning to provide and the actual care being provided. The overall interpretation of the studies produces priority areas of research as setting priority for compliance rather than simply attempting to raise average levels, measuring outcome of care which are free of predetermined ideas and have high face validity. Quality Assessment (QA) of STI case management is an effort to upgrade quality of STI diagnosis and treatment services delivered by the AIDS-STD clinics in the country. Furthermore, quality STI services can promote utilization of STI services provided by the clinics. In addition, the use of STI services can be either an entry point for prevention of further sexual risk behavior or a point of access (through partner referral) to other people at risk of HIV and STIs. In order to achieve these objectives quality assessment studies are introduced and the results are documented. Quality assessment of STI care is the first attempt to gain experiences on assessment methods and clues for improvement of actual STI case management services. Objectives of the study were;

- To measure the percentage of patients with STIs at selected health care facilities who were appropriately diagnosed and treated according to national guidelines, of all STI patients at those centers (STI Service Indicator 1)
- To estimate percentage of patients with STIs who were given advise on condom use and partner notification and who were referred for HIV testing (STI Service Indicator 2)
- To assess adequacy of drugs and equipment in STI care facilities currently providing care (STI Service Indicator 3)

MATERIALS AND METHODS

Cross-sectional study design was used. The assessment methods and tools included direct observation on patient-provider interactions, reviewing patients' records and registers for essential STI drugs available at the clinics and facility inspection by using observation checklist. Face-to-face and exit interviews were conducted with healthcare providers (HCP) and patients by using pretested unstructured and structured questionnaire. Myanmar Standard Treatment Guidelines for the Management of STIs and draft UNAIDS/WHO methods package for STI care and prevention assessment in evaluation of NAP were used as standards for assessing quality of STI case management. STI service indicators 1, 2 and 3 were measured to assess quality of STI case management [5, 6]. Enumerator training was conducted for obtaining informed consent, interpersonal interviewing and observation skills; recording observations on a standard sheet. Data collection started in December 2006 and ended in February 2007.

With the consultation of the NAP, a sample of seven clinics was drawn from 45 clinics all over the country by stratification. AIDS-STD clinics included in the sample were Lashio, Chanayethazan (Mandalay), Monywa, Pakokku, Kyimyindaing (Yangon), Patheingyi and Mawlamyaing. The reasons for selection of these clinics were: (1) they are strategically located in the country (2) STI patient volume is fairly large and (3) facilities are fully staffed and equipped according to national standards. As a total number of STI patients with genital symptoms attending the facilities daily were around five, all were included in the sample according to the guidelines of Health Facility Survey [6]. Study populations were management of 47 STI cases conducted by 14 health care providers including doctors (STD team leaders), nurses and health assistants from study clinics. All drug stores were selected from seven AIDS-STD clinics to measure the availability of essential STI

drugs. After checking consistency and completeness of the questionnaire and checklists, data entry, processing and analysis were done by using EPI-INFO and SPSS software.

RESULTS

A total of 47 STI case managements in seven AIDS-STD clinics were observed. It was found that 75% of case managements were done by doctors, 19% were done by nurses and 6% were done by health assistants. Mean age of STI patients was 30 years with standard deviation of 10 years. The youngest patient was 18 years old and the oldest patient was 55 years old. Forty-three percent of patients were males and others were females. Forty-five percent of patients were married and 68% of patients had history of commercial sex.

History taking

Regarding the STI case management, quality assessment was done on proper history taking, physical examination, diagnosis, treatment, educational counseling and follow-up. Interrogation about the illness was an important step taken by HCP during the patients' visit to the clinic. According to the national guidelines of the STI case management, a particular provider should ask three important items to the patients, i.e. nature of presenting symptoms, onset or duration and recent sexual contact. In the study, it was found that HCP asked nature of presenting symptoms in all case managements, onset or duration of symptoms in 94% of case managements and history of recent sexual contacts in 70.2% of case managements. Therefore, it was estimated that only 33 patients (70.2%) received adequate history taking. Main STI symptoms presented by patients were genital discharge (47%) and ulcer/warts (36.5%). Male STI patients mostly presented with genital ulcer, urethral discharge and female patients mainly presented with white discharge per vagina.

Physical examination

Physical examination was second step taken by the providers in the STI clinics and it was found that 11 patients (23.4%) received proper physical examination. As some patients had double or triple pathology, there were 57 diagnoses made by HCPs. Out of 57 diagnoses, 44 (77.2%) were diagnosed by etiologic basis and 13 (22.8%) were diagnosed by syndromic basis. Detailed findings are shown in Table 1. Laboratory investigations done to obtain etiologic diagnosis were Gram stain smears, dark field microscopy, VDRL tests and HIV test. Gram stain smear examinations were done for 16 patients (34%) and only one patient received results on the day of examination. VDRL test was done for 39 patients (83%) and 8 patients (17%) received results on the day of consultation. Eleven STI patients (23.4%) also carried out HIV testing.

Table 1. Number and percentage distribution of STIs diagnoses made by service providers

Type of STIs diagnosed (n=57)	Number	Percentage
<i>Etiologic diagnosis</i> (n = 44) (77.2%)		
Gonorrhoea	8	14
Syphilis	12	21.1
Genital warts	6	10.5
Chancroid	4	5.3
Herpes simplex virus	2	3.5
Candidiasis	10	17.5
Genital scabies	2	3.5
Genital ring worm	1	1.8
<i>Syndromic diagnosis</i> (n = 13) (22.8%)		
Urethral discharge	6	10.5
Vaginal/cervical discharge	6	10.5
Genital ulcerative disease	1	1.8
Total	57	100

Effective treatment

Out of 57 diagnoses, it was found that 32 patients (56.2%) received appropriate treatment for the diagnoses. Types of treatment given by providers are shown in Table 2.

STI service indicators

STI service indicator 1 measures appropriate diagnosis and treatment of STI. It is defined

Table 2. Types of treatment given by providers

No.	Type of treatment (n=57)	Number	Percentage
1	Effective syndromic treatment	3	5.3
2	Effective etiologic treatment	29	50.9
3	Syndromic treatment given to etiologic diagnosis	15	26.3
4	Ineffective etiologic treatment	1	1.8
5	Ineffective syndromic treatment	9	15.7
6	Received appropriate treatment	32	56.2

as percentage of patients with STIs or STI symptoms at selected health care facilities who are appropriately diagnosed and treated according to national guidelines, among all STI patients seeking care [6]. Assessment was done by giving scores to the case management of STIs if HCP followed each component of national treatment guidelines as score 1 and for those where HCP did not follow were given score 0. Therefore, a case of STI where an HCP conducted the adequate history taking, proper physical examination and providing correct diagnosis and treatment received a total of score 3. In this study, it was found that only one case management (2.1%) received score 3, 51% of case managements received score 2 as HCP did not meet the criteria for proper physical examination, 36% received score 1 and 11% received score 0. Direct observation of patient-provider interactions revealed the reasons for failure to practice proper physical examinations: low privacy of examination rooms, inadequate use of equipment such as spot lights and speculums etc., lack of interest of some providers and assigning inappropriate provider to do physical examinations e.g. assigning female provider to male STI patients.

STI service indicator 2 measures providing advices to STI patients for prevention and referral to voluntary testing services. It is defined as percentage of patients with STIs or STI symptoms who are given advice on condom use, partner notification and who are referred for HIV testing, among all STI patients seeking STI care [6]. In this study it was observed that out of 47 patients, 25 patients (53%) received explanation for risk

of HIV infection, 28 (60%) received advice on condom use and 12 (30%) received advice on partner notification. Out of 42 patients who needed referral for HIV testing only 18 patients (43%) received referral for HIV testing. It was also found that 21 STI patients (45%) received condoms free of charge and 17 patients (36%) received instructions on how to use condom.

In actual assessment, score 1 was given to the STI case management where an HCP provides each item of service according to the National Standards. Therefore, each case management where HCP provides all three advices will receive a total of score 3. In this study, it was found that 8.5% of STI case management received score 3, 34% received score 2, another 34% received score 1 and 23% received score 0. Detailed findings of scores obtained for STI service indicator 1 and 2 are shown in Table 3.

Table 3. Scores for STI service indicator 1 & 2

No.	Type of score	STI service indicator1 STI service indicator2			
		Case management		Case management	
		Number	Percent	No.	Percent
1	Score 0	5	10.6	11	23.4
2	Score 1	17	36.2	16	34.0
3	Score 2	24	51.1	16	34.0
4	Score 3	1	2.1	4	8.5
		47	100	47	100

STI service indicator 3 measures the adequacy of drug supply at STI clinics. It is defined as percentage of clients served by STI facilities that have a current supply of all essential STI drugs and report no stock-outs lasting longer than one week in the preceding 12 months, among all STI patients seeking care [6]. It was found that no single clinic had all 13 items of essential STI drugs. Doxycycline was the only essential drug available in all clinics with no stock-outs during the last 12 months. All other essential drugs were having stock-outs lasting longer than one week in the preceding 12 months. Therefore, all STI facilities could not accomplish the criteria set by STI service indicator 3.

Availability of drugs

Ensuring adequate supply of essential drugs used for STI case management is important for effective treatment of STIs and to have positive impact on reducing the likelihood of HIV infection. It was observed that no clinics had all essential STI drugs (13 items) and ten essential STI drugs were available at 3 (43%) clinics only. The least available drugs that were unexpired in the clinics were meconazole tablet, ceftriaxone injection, nystatin vaginal tablet and podophylline lotion. Regarding management of STI drugs, it was found that AIDS-STD team leader took responsibility for procurement and use of drugs and supplies in all studied clinics. Number and percent of clinics where essential STI drugs available are shown in Table 4.

Table 4. Availability of essential STI drugs in AIDS-STD clinics

No.	Name of drug	Clinics*		Clinics**		Clinics***	
		No	%	No	%	No	%
1	Inj. Ceftriaxone	2	28.6	5	71.4	2	28.6
2	Cefixine	6	85.7	2	28.6	5	71.4
3	Azithromycin	5	71.4	3	42.9	4	57.1
4	Doxycycline	7	100	0	0	7	100.0
5	Erythromycin	6	85.7	1	14.3	5	71.4
6	Ciprofloxacin	4	57.1	3	42.9	3	42.9
7	Metronidazole	6	85.7	1	14.3	6	85.7
8	Meconazole	2	28.6	5	71.4	1	14.3
9	Inj. Benzathine Penicillin	6	85.7	2	28.6	6	85.7
10	Nystatin Vag. Tab	3	42.9	4	57.1	2	28.6
11	Clotrimazole	5	71.4	3	42.9	4	57.1
12	Acyclovir	7	100.0	2	28.6	7	100.0
13	Podophylline	3	42.9	5	71.4	2	28.6

* where STI drugs are available at the time of observation

** where STI drugs are not available for at least one week in the last 12 months

*** where unexpired drugs were observed

DISCUSSION

The study on quality assessment of care of STIs in the AIDS-STD clinics was aimed to improve quality of services provided at these centers and to control the spread of STIs in the general population. As there

were limited resources to conduct the study, the number of facilities and subjects in the sample was smaller than other studies [7]. However, seven STI clinics were selected in the sample to reflect the situation of STI in hilly, central dry zone and delta regions. Furthermore, AIDS-STD clinics from cities as well as from states and divisions were selected in the sample to represent the real country situation as much as possible. In this study, STI indicators 1 to 3 developed by UNAIDS were used for assessment of STI case management services [4, 6]. These indicators are very similar to the original WHO indicators PI6 and PI7 [1] with the addition of referral for voluntary counseling and testing (VCT) and STI indicator 3 measuring STI drug stock-outs. Furthermore, study design, data collection methods, instruments and sampling methods were developed according to the UNAIDS guidelines of protocol developed for assessment of STI case management through health facility survey [6]. Only "Mystery client interviews" could not be carried out due to technical and resource constraints.

Assessment of STI indicator 1 focused on the proper history taking, physical examination, diagnosis and treatment. According to the study findings, a considerable number of patients did not receive proper physical examination resulting more than half of the case managements received score 2 during assessment. The reasons of failure to practice proper physical examinations were mentioned as observation findings. Therefore, quality of physical examination is questionable especially in the case of male patients consulting to female provider. One remarkable finding of the study was majority of providers made etiologic diagnosis in spite of doing only a few laboratory investigations. The reason of this situation might be low availability of laboratory services or HCPs making empirical diagnoses mostly. Another finding was providers could give more effective etiologic treatment than more effective syndromic treatment. Some providers gave syndromic treatment to the patients with

etiologic diagnosis. This might be due to the providers' behavior that tended to over-treat the STI cases. As a result of these flaws, HCPs who could make appropriate diagnosis and treatment of STIs were very low.

STI service indicator 2 was assessed by observing the consultations with HCPs. We found that only 4 patients (8.5%) received adequate advices and referral to VCT. Major areas of advice that did not touch by HCPs were explaining risk of HIV infection, explaining how to use condom and advice on partner notification. Drug stock-outs in the STI clinics are also a problem. It was observed that more than a half of clinics had shortages of three or more items of drugs and surprisingly only three drugs were available in one clinic. Therefore, it is important to find the causes of stock-outs and correct swiftly because frequent stock-outs of drugs may lead to under-utilization of STI services. Based on the findings of the study the following recommendations are made:

- (1) As appropriate diagnosis and treatment are the keys to the achievement of STI case management objectives, HCPs in the STI clinics should do systematic history taking, physical examination, diagnosis and treatment. The study also highlights the factors that lead to low quality of services such as low privacy of examination rooms, inadequate use of equipments, lack of interest in physical examinations by some HCPs and assigning inappropriate HCP to the STI patients. The causes or contributing factors of these conditions should be thoroughly analyzed and prompt actions should be taken.
- (2) Inappropriate treatment of STIs by HCPs should be corrected by training and supervision. Trainings of STI case management should be supported by regular supervision of health care providers whether they are following the National Standard Treatment Guidelines for the Management of Sexually Trans-

mitted Infections or not. As etiologic treatment is more effective than syndromic treatment, provision of etiologic treatment should be strengthened with adequate support of laboratory facilities.

- (3) Advice on condom use should be targeted to all STI patients. Further more, effective strategies should be developed to upgrade partner notification and referral for VCT.
- (4) The study also indicates frequent STI drug stock-outs and expiries in the clinics, which impedes the achievement of STI case management objectives. This problem should be solved by reviewing drug procurement, supply and distributing systems and taking appropriate actions.

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**Local Non-Governmental Organizations (NGOs)
participation in National Tuberculosis Programme (NTP)**

**Tin Tin Wynn, *Maung Maung, **Bo Myint, *Khin Wai & *Zaw Win Tun*

**Health System Research Division
Department of Medical Research (Upper Myanmar)
**Tuberculosis control, Mandalay Division*

Tuberculosis (TB) is one of the priority health problems in Myanmar. The objectives of this study were to identify the types of local Non-Governmental Organizations (NGOs), to determine the areas of participation in National Tuberculosis Program (NTP) and to determine the association between involvement of NGOs and achievement of NTP. A descriptive cross-sectional community-based study was conducted. Pyigyitagon, Nyaung U, Wundwin and Myingyan Townships were selected. Study period was from Nov 2006 to July 2007. For quantitative survey, 325 new sputum smear positive TB patients and 60 NGO members were studied. Focus group discussions with NGOs were done. Types of NGOs were Myanmar Maternal and Child Welfare Association (60%), Myanmar Women's Affair Federation (31%) and Red Cross (9%). Areas of participation were diagnosis (18.8%), referral of TB suspect (44.0%), provision of DOTS (12.6%), financial support (8.1%) and others (defaulter tracing, health education) (16.5%). About 33% of NGOs were good DOTS providers. The better the case detection (R^2 0.9623) was observed in the townships with more NGOs participation. Strongly positive association between NGOs involvement and achievement of NTP was found. NGOs can make an important contribution by facilitating links between health services and local community.

INTRODUCTION

Effective tuberculosis (TB) treatment delivery in high-burden countries requires a multisectoral approach. The participation of local Non-Governmental Organizations (NGOs) in Directly Observed Treatment Short-course (DOTS), TB care delivery through primary health care facilities is one of the innovative ways. For TB control, the need to involve other stake-holders such as community and NGOs is not new and it was well recognized by WHO (1974), which stated; "It is important that the community should be involved in the TB program, including its leaders such as village elders, tribal chieftains, or other influential persons and the welfare organizations including the voluntary agencies and the laity" [1].

In the public health literature, there is a paucity of evidence on local NGOs involvement in community-based TB program despite there was the potential of these organizations to improve the accessibility of services [2]. Government identified local NGOs as potentially useful partners that can assist the TB program to implement DOTS. NGOs participate in the TB program by providing case finding, referral of TB suspect, DOTS provider, defaulter tracing and other social supports. This paper seeks to describe the involvement of local NGOs in TB control effort in four townships of Mandalay Division, Myanmar.

Objectives

- To identify different types of local NGOs actively participated in TB control program

- To determine the area (finding of TB suspect, diagnosis, administering TB drugs under DOTS, follow-up, defaulter tracing, social supports) of TB control actively implemented by NGOs
- To determine an association between active involvement of NGOs and achievement of NTP

MATERIALS AND METHODS

The present study was conducted in Pyigytagon (PGTG), Nyaung U (NU), Wundwin (WD) and Myingyan (MG) Townships, Mandalay Division, Myanmar which were chosen purposively (according to township TB patients register there was more than one hundred new sputum smear positive patients per year).

Study period

From September 2006 to August 2007

Inclusion criteria for TB patient

- New sputum smear positive patient registered in township TB campaign within one year
- Treatment completion of 6 months duration
- Both sexes
- Age above 12 years
- Pure TB case

Data collection

According to inclusion criteria, sputum smear positive adult patients with completion of treatment (n= 325) were randomly selected from patient register and list of NGOs involved in TB control was collected from township TB control team leader (n= 60) and they were interviewed with semi-structured questions by trained interviewers. The questionnaire for TB patients included type of help received from NGO, source of information about TB, health education activity and recording and keeping systems of treatment card were obtained.

In study townships, selected members of NGOs who participated in TB control were

invited for face-to-face interview and focus group discussion (FGD). During survey period, some NGOs could not take part in study for interviewing because of their personal matters, however, they participated in control activity. In Myingyan township, campaign assigned family member of TB patient as DOTS provider, therefore only NGO who was family member of TB patient became provider. In other townships, not only family member but also other person can perform as provider such as NGO, health person etc.

Based on township variable such as Pyigytagon, Nyaung U, Wundwin and Myingyan, a total of 8 FGD sessions (2 sessions in each group) were conducted with the selected NGO members. While all NGO members had experience in TB control activities, one NGO in Myingyan Township (Pyigyikhin) had also dealt with TB co-infected AIDS patients. Information relating to perceptions towards TB and NTP, NGO involvement in NTP and community perception on involvement of NGO on NTP were gathered through FGDs. Reviewing of existing records and reports of townships was conducted. All townships except Myingyan Township members of NGOs participated in TB control more than three years duration. Hence, case detection rate, treatment completion rate and cure rate were reviewed for three years duration (2003-2005) in all townships.

RESULTS

General characteristics of respondents

The general characteristics of TB patients who registered and took treatment in respective study townships are shown in Table 1.

Types of NGO

This study tried to elicit the types of NGO participating in TB control program in the study townships. Three NGOs, namely Myanmar Maternal and Child Welfare Association (MMCWA), Myanmar

Women's Affair Federation (MWAF) and Red Cross (RC) participated in TB control program. The participation of NGOs varied from townships to townships. While all the

Table 1. Age and sex distribution of TB patients by townships

Charac- teristics	Townships				Total
	PGTG	NU	WD	MG	
	No. (%)	No. (%)	No. (%)	No. (%)	
Age (Yrs)					
10-19	25(44.7)	11(21.3)	10(19.1)	9(14.9)	55(16.9)
20-29	16(21.9)	16(21.9)	21(28.8)	18(27.4)	71(21.9)
30-39	18(26.1)	15(21.2)	17(24.6)	21(27.1)	71(21.9)
40-49	12(22.4)	11(20.7)	11(20.7)	21(36.2)	55(16.9)
50-59	12(25.6)	19(41.9)	6(11.6)	9(20.9)	46(14.2)
60-69	7(34.6)	5(23.1)	3(19.2)	5(23.1)	20(6.2)
70+	0 (0)	4(4.4)	1(22.2)	2(33.4)	7(2.2)
Sex					
Male	50(23.6)	59(28.1)	41(19.5)	60(28.8)	210(64.6)
Female	38(38.8)	22(19.1)	30(26.9)	25(20.2)	115(35.4)
Total %	88(27.1)	81(24.9)	71(21.8)	85(26.2)	325

PGTG = Pyigyitagon, NU = Nyaung U, WD = Wundwin
MG = Myingyan

NGO members from Nyaung U involved in TB control activities, i.e., MMCWA was 22.5%, MWAF was 36.8 % and RC was 40.7%, only MMCWA from Pyigyitagon and MWAF from Wundwin participated in TB activities respectively. However, there were no NGOs member involvement in control program in Myingyan Township because during study period found that TB campaign assigned family member as care providers who were accompanying during visit for anti-TB treatment. Therefore NGOs act as care providers who were family member of TB patient.

Areas of participation

This study tried to find out the areas of TB control program (Objective 2) such as case detection, referral, case holding or DOTS provision, and defaulter tracing by asking the TB patients how NGO was involving in TB control program (Table 2). It was noted that 165 TB patients (50.8%) responded that they got help from NGO in different aspects

during TB treatment. In other words, one out of every two TB patients received help from NGO in a form of either cash or kind.

Table 2. Areas of participation by NGOs

Areas of participation	Number of NGOs	Percentage
Diagnosis	20	6.2
Referral	75	23.1
DOTS provider	49	15.1
Financial support	5	1.5
Others	16	4.9
Nil	160	49.2
Total	325	100

The respondents reported that they received other types of help from NGOs as well; namely 12.2% admitted they got help in diagnosis, 45.5% in referral, 29.7% in DOTS provider, 3% in financial support and 9.6% in other (health education concerning TB and defaulter tracing) activities. It was found that NGOs involved in each and every areas of TB control program. However, the involvement of NGO in study townships was only 50%. Nevertheless, they gave financial support to the poor.

Quality of DOTS by NGO

The study also accessed the quality of DOTS provision activity in 49 patients who mentioned receiving the help from NGO as DOTS providers according to WHO definition i.e. provider gave daily dose of anti-TB drugs to patients and watched them until they have swallowed the drugs (Table 3).

Table 3. Quality of DOTS by NGO

Quality of DOTS	No. of cases	Percentage
Provide wholesome drugs, monthly or weekly	8	16.30
Give daily dose in hand without watching the act of swallowing	25	51.00
Provision of drugs daily and Watching the act of swallowing	16	32.70
Total	49	100

Some 32.7% (16/49 TB patients) received help from NGO members acted as DOTS providers. They gave the daily dose to the patients and watched them until swallowing of drugs. However, 51% (25/49 TB patients)

said, NGO just gave the daily dose of drugs to the patients without watching the swallowing. The remaining 16.3% (8/49 TB patients) were provided with the drugs monthly or weekly without observing the swallowing. In short, only one third of NGO DOTS providers were reliable and strictly adhered to WHO criteria of DOTS provider.

Constraints of NGO members

Findings from focus group discussion revealed that distance between the residence of patients and NGO member was one of the constraints to watch the act of swallowing. The drugs were already taken by the patients by the time they arrived at patient's home, if the patient's residence was too far from them. However, NGO members strictly followed the criteria only for those patients who lived nearby.

Association between NGO participation and achievement of NTP

This study tried to find out the association between NGO participation and success of NTP (Objective 3). The indicators for the success of NTP were Case Detection Rate (CDR), Treatment Completion Rate, Cure Rate and Defaulter Rate. These secondary data were obtained from the Divisional NTP office (formerly called Zone II) and correlated with number of NGO involvement in respective townships. Among these data, only case detection rate was used to assess correlation between achievement of NTP and degree of involvement of NGO in the form of number of NGOs actively participated in TB control program (Table 4).

Table 4. Correlation between number of patients received help from NGOs and case detection rate of respective township

Townships	NGO's participation*	Case detection rate	Correlation value	P value (2-tailed)
Pyigyitagon	80.68 (71/88)	99	0.959	0.184
Nyaung U	37.04 (30/81)	56		
Wundwin	43.66 (31/71)	74		

* Numbers of patients referred by NGO divided by total detected cases in each township

The linear regression model was applied and found that the more participation of NGO

members produced higher case detection rate and better achievement of TB control program.

DISCUSSION

Many Asian countries have a long experience of NGOs contribution in TB control [3, 4]. However, population and area coverages are quite different from country to country. Unlike the NGOs in our country, NGOs from India and Bangladesh are small associations representing the whole town or part of the region such as SEWA (Self Employed Women's Association), which covered city population and hospital-based REACH (Resource Group for Education and Advocacy for Community Health) covering 294 women's groups and HEED (Health Education and Economic Development) mobilizing women's group in Bangladesh.

In addition, nature of NGO involvement in TB control program is not the same in all countries. NGO in India hands over the whole process of NTP activities – diagnosis, treatment and follow-up. In other words, in India, NGO acts as NTP whereas in Myanmar, NGO supports the NTP focusing on supportive humanitarian work rather than medical activities. On the other hand, activities done by NGO in Bangladesh and Myanmar are quite similar in the areas of identification of symptomatic and referral to the diagnostic centres, direct observation of treatment DOT, default retrieval, collection of sputum sample from the symptomatic and health education about TB. However, it is more extensive in Bangladesh.

The NGO involvement in NTP is also one of the contributing factors to the achievement of NTP. Depending on the area or kind of activities involvement by NGO, the achievement was also different. In India and Bangladesh studies, NGO involved in particular area; while increase in out-patient attendance and case detection were the result of referral of symptomatic, increase in cure rate was due to monitoring of drugs consumption and defaulter retrieval. In

present study, NGO participation was more focused on referral of TB suspect (45.4%-75/165 patient), which reflected in case detection rate of 151.2% in Pyigyitagon with more NGO participation and 48.3% in Myingyan with no NGO participation. Even that, NGO in Myanmar neither receive training concerning TB nor incentive. They got only acknowledgment from the patients.

An intervention study done in Myanmar showed that by using trained MCWA as DOTS provider in study area, case detection rate and success rate were higher in study area than that of in control area [5]. Similarly, in Tamilnadu India, the whole province (with or without NGOs involvement) increased in case finding by 20%, treatment completion rate of 83% and cure rate of 82% to 93% were observed. So also, it was found that participation of NGO contributed to increase in cure rates in Bangladesh and Philippine [6].

Despite the achievement, NGO from the study area encountered with some difficulties such as overburden because of engaging with several health-related work, delaying in work commitment due to lack of monetary rewards, poor comprehensive skill due to low educational attainment, inadequate training of NGO and relatively weak system of monitoring and supervision [7, 8]. Although this finding could not be generalized for the whole country, to some extent, this indicated the need to strengthen treatment observation (DOTS provider activity) and completion of TB patients, which could not be done by health staffs alone, community as well as NGOs should participate hand in hand with health personnel.

Recommendations

- Wider NGO participation should be strengthened in TB control than present situation.
- NGO participation should be present in every township.

- Apart from MMCWA, MWAFA and RC other NGOs should also participate in NTP.
- Should improve and strengthen the quality DOTS provider activity more than 32% by reducing the constraints and difficulty e.g., patient and NGO ratio should be 1:1.

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Factors influencing the performance of Health Assistants in selected townships, Myanmar

*Kyaw Kyaw, *Thida & *Maung Maung

*Epidemiology Research Division
Department of Medical Research (Upper Myanmar)

A cross-sectional study was conducted to determine factors influencing the performance of Health Assistants (HAs) from May 2006 to July 2007 in twenty-three townships of plain and hilly regions of Myanmar. Out of 79 HAs, 61 (77.2%) males and 18 (22.8%) females were interviewed with structured questionnaire. Mean age of HAs, mean years of service and mean distance from township hospital were 41 ± 9 years, 15 ± 11 years and 15 ± 9 miles respectively. Both intrinsic and extrinsic factors were asked. Regarding intrinsic factors, positive response rates of perception, satisfaction, willingness, attitude and motivation were observed at 68.4%, 68.4%, 92.4%, 65.8%, and 92.4% respectively. Extrinsic factors like locality of Rural Health Center (RHC) (plain or hilly region), transportation to RHC and public transportation from RHC to township hospital were associated with performance of HA and it was statistically significant at $p < 0.05$. Reconsideration should be made on deployment of resources to upgrade infrastructure in health system especially in rural area to attain effective performance of assigned health staff.

INTRODUCTION

Health Assistant (HA) is the key person among Basic Health Staff (BHS). At the village level, team composes of BHS, headed by HA carries out Primary Health Care including both public health and disease control. Under each Rural Health Center (RHC), there are four sub-centers staffed by midwives and Public Health Supervisors Grade 2 (PHS II) [1]. Nearly 70% of people living in rural areas can seek health care from Rural Health Centers (RHCs) or Station Hospitals run by HA and Station Medical Officers (SMO) respectively. Besides, HAs are leading midwives and other health staff energetically in carrying out health tasks like health education, environmental sanitation, communicable diseases control, maternal and child health care, school health nutrition, birth and death registration and medical care. The term performance is meant to focus on the behavior of a health

worker including his organization retention and use of specialized knowledge as well as his attitude and interaction with other people. However, this may be different depending on skill, motivation, geographical locality and available resources. Thus, assessing the performance of HA in different localities of the country would be applicable in health management system by the health planners and authorities.

General objective

- To find out the factors influencing the performance of HAs which can be applicable to health management system to produce sustainable improvement.

Specific objectives

- To access the performance of HAs in different areas of primary health care in the assigned areas.
- To determine the factors that influence the performance of HAs assigning specific jobs.

MATERIALS AND METHODS

Study design

A cross-sectional, descriptive study was applied.

Study population

A total of 79 Health Assistants posted at various health centers of the country were included

Study areas

Twenty-three townships of various states and divisions where the selected 79 HAs were posted, namely: Myitkyina, Winehmaw and Moekaung from Kachin State; Hakha, Htantalan and Phalan from Chin State; Thenni, Hsipaw and Naungcho from Northern Shan State; Taunggyi, Hopone and Naungshwe in Southern Shan State; Sagaing, Myinmu, Chaungoo and Myaung from Sagaing Division; Magwe, Minbu, Yenanchaung and Chauk townships from Magwe Division; Kyaukse, Yamethin, and Meikhtila from Mandalay Division.

Data collection

Face-to-face interview with a set questionnaire was undertaken. Checklist was also developed for assessing performance of HA. Observation of RHC structure and functional analysis was done. Secondary data analysis of reports and returns of RHC was conducted. Performance was assessed by checklist developed from the tasks rendered by the HA, based on seven categories of health functions assigned by the government. Based on the activities performed by the HA, good and poor performances were categorized. Both extrinsic and intrinsic factors regarding HA performance were asked with the pre-tested questionnaire. For factors influencing HA performance, two factors, extrinsic and intrinsic were added. Assessment of locality of RHC and accessibility to RHC were defined as extrinsic factors. Perception, willingness to work, attitudes and motivation of HA were assessed for intrinsic factors. Observation of RHC was performed to validate the answer

by HA obtained from the questionnaires. Reports and returns of respective RHC were also reviewed to check the consistency of the answers.

Data management and analysis

Data were entered, stored, and managed by Epidata. Data cleaning and consistency check was done. Crude odds ratio (OR), adjusted OR were calculated by Epi-info and multivariate analysis by STATA version 8.

RESULTS

A total of 38 villages were served by one HA and the population coverage was found to be 27,206. Socio-demographic characteristics of HA were reviewed and it was found that 77.2% were males whereas 22.8% were females. Most (63.3%) resided away from RHC and only 36.7% were living in the same locality of RHC. Regarding educational status, 63.2% were graduated and 36.8% were high school level. Mean age of HAs was 41 ± 9 years with a range of 23 to 58 years. Mean year of service was 15 years ranging from 3 to 38 years. The mean distance from township to RHC was 15 ± 9 miles with a range of 2 to 40 miles.

Table 1. Locality of RHC and accessibility

Particulars	Number (%)
<i>Locality of RHC</i>	
Plain region	60 (75.9)
Hilly region	19 (24.1)
<i>Transportation to RHC</i>	
Available	50 (63.2)
Not available	29 (36.8)
<i>Public transportation from RHC to township hospital</i>	
Accessible	58 (73.4)
Inaccessible	21 (26.6)

In this study, majority of RHCs (75.9%) are located in plain region and 24.1% are in hilly region (Table 1). Utilization of RHC was mainly influenced whether it has been easily accessible by means of walking, using bicycle or bullock carts etc. It was found that 63.2% of RHC were in localities where the community can reach easily. For

referral cases the community relies on public transportation. Therefore, public transportation is a key factor for utilization of RHC and it also indirectly influences the performance of HA. This situation was evident in immunization activities. If the location was easily accessible there would be more coverage of immunization.

Factors influencing performance

Factors influencing performance were assessed by intrinsic and extrinsic factors. For intrinsic factors, job related perception, satisfaction, willingness to volunteer, and motivation were included. For those intrinsic factors all categories were found to be good at minimum 65.8% to maximum 92.4% (Table 2).

Table 2. Perception, satisfaction, willingness, attitude and motivation of HAs

Intrinsic factors	Number (%) (n=79)	
	Good	Poor
Perception	54 (68.4)	25 (31.6)
Satisfaction	54 (68.4)	25 (31.6)
Willingness	73 (92.4)	6 (7.6)
Attitude	52 (65.8)	27 (34.2)
Motivation	73 (92.4)	6 (7.0)

Regarding extrinsic factors, localities of RHC, accessibility to RHC and transportation from RHC to township hospital were analyzed. Chi-square test was applied. Table 3 indicates that there is association between extrinsic factors and performance of HA in all study areas.

Table 3. Performance of HA by extrinsic factors

Factors	Performance (n=79)			Adjusted OR
	Poor	Good	Total	
<i>Locality of RHC</i>				
Plain region	40	21	61	4.42
Hilly region	17	1	18	p=.01
<i>Transportation to RHC</i>				
Easy to reach RHC	35	19	54	3.49
Not easy to reach RHC	22	3	25	p=.03
<i>Accessibility of transport from RHC to hospital</i>				
Accessible	36	19	55	3.02
Not accessible	21	3	24	p=.04

Association between job performance and localities of RHC (plain or hilly region) was statistically significant. Therefore, performance of HA in plain region was 4 times better than that of HA in hilly region. Performance and availability of transportation to RHC was also significantly associated. Consequently, performance of HA in area where transport is available to RHC was 3 times better than that of HA in area where transport is unavailable to RHC. Association between performance and public transportation from RHC to township hospital was statistically significant as well. This indicated that for the RHC where the public transportation was accessible, performance of HA was 3 times better than that of HA in the RHC where public transportation was inaccessible.

DISCUSSION

A total of 79 HAs working in various health centers of 23 townships in plain and hilly region were included in this study. According to a report from Turkey, 60 % of health care workers were satisfied with their jobs. Working environment and income were the most important factors for dissatisfaction. Health care workers at public health centers in the provinces have low satisfaction scores. Improving working conditions and income may improve the overall quality of health care provision [2].

Manongi *et al.* reported that factors causing de-motivation among health care workers working at primary health care facilities were workload, staff shortages, lack of inter-professional exchange, and lack of supervision [3]. Nilar Tin *et al.* highlighted that 67% of BHS were satisfied in works. Although some complained on a lot of paper work, majority of BHS agreed that they enjoyed field work, giving services including health education [4]. In this study, the performance of HA was not influenced by the intrinsic factors like perception, satisfaction, attitudes and motivation of the study group. It seemed that the HA have

positive attitude towards performing health activities by revealing response rates of perception, satisfaction, willingness, attitude and motivation at 68.4%, 68.4%, 92.4%, 65.8%, and 92.4% respectively. The intrinsic factors were not associated with performance of HAs in this study. But there was an association between extrinsic factors like locality of RHC (plain or hilly region), transportation to RHC and public transportation from RHC to township hospital and performance of HAs which was statistically significant. Despite a series of work study and performance of health system in Myanmar, there is still a gap of knowledge on providing extrinsic and intrinsic factors influencing health staff's performance. The findings of this study provided that extrinsic factors need to be highly considered and reconsideration should be made for upgrading infrastructure to attain effective performance of health staff especially in rural area.

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Treatment compliance and knowledge of hypertensive patients in Hlinethaya Township, Yangon Division

Sandar Kyi, *Han Win, *Aung Thu, *Yae Chan, *Myat Myat Thu, *Khin Thidar Wai, *Kyi Sann Htet & **Khin Myat Tun*

**Clinical Research Division*

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

****Department of Health*

A cross-sectional survey was conducted on 133 hypertensive patients in Hlinethaya Township, Yangon Division in May 2007. The aim of the study was to determine the treatment compliance of hypertensive patients, their knowledge and misconception about hypertension. The mean age of the participants was 54.67 ± 12.77 years. Compliance was measured by therapeutic outcome method where blood pressure less than 140/90 mmHg was considered as the indicator of treatment compliance. Compliance with treatment was observed in only 29% of the respondents. The compliance was better in those aged <45 years than older patients (48.5% Vs 9.4%; $p < 0.01$). Among the respondents, 60.2% believed that they could stop drug treatment once they achieved blood pressure control. Although 34.6% of respondents were aware that too much salt intake was an etiological factor in hypertension, 43.6% did not know the complications of hypertension. The findings indicate that there is a need for health education to enhance the compliance and knowledge about the disease among hypertensive patients in the study area.

INTRODUCTION

Hypertension is an important public health problem of global dimensions, both in developed and developing countries. It has been estimated that about 20% of adult population have high blood pressure worldwide [1]. High blood pressure contributes to the leading cause of morbidity and mortality from coronary artery disease and stroke [2, 3]. Although safe and effective drugs are available, the management of hypertension is still far from optimal especially in the developing countries [4].

According to a previous cardiovascular diseases survey in Myanmar, the prevalence of hypertension was 12.5% and 14.6% in rural and urban areas respectively [5]. A previous study conducted in Hlinethaya Township by diabetes mellitus control project, Myanmar revealed that the prevalence of hypertension in that area was 35%

(Personnel communication). A major factor accounting for inadequate control of hypertension is poor compliance with treatment [6, 7, 8]. Compliance was considered as the adherence to medical advice about life-style modification as well as to keeping appointments for follow-up and taking treatment as prescribed. Poor compliance is especially common in the patients with poor knowledge, understanding, and perception of hypertension. As there was no data on treatment compliance and knowledge of hypertension in Hlinethaya Township, Yangon Division, this study was carried out with the following objectives.

Objectives

- To determine the treatment compliance of hypertensive patients in Hlinethaya Township, Yangon Division
- To find out the hypertensive patients' knowledge and misconception about the disease

MATERIALS AND METHODS

Study area and population

The study was conducted in Hlinethaya Township, Yangon Division in May 2007. A cross-sectional descriptive study design was used. An urban area of Hlinethaya Township, Yangon Division was purposely selected and five wards from that area were selected by using probability proportionate to sampling (PPS). Persons who had hypertension for at least 6 months were identified through health staff and members of Ward Peace and Development Council (WPDC) of selected wards before the survey. Then subjects were invited to come to the office of WPDC. From each ward, subjects were taken consecutively till the required sample size was accomplished.

Sample size determination

It was assumed that crude prevalence of hypertension in community was 15% and compliance of treatment among hypertensive patients was 50%. Therefore, the proportion of hypertensive patients with proper compliance was 7.5% and desired precision was set at 0.05, the required sample size were about 106. In this study, a total of 133 subjects were recruited in order to cover the non-response rate.

Methodology

Before the data collection, informed consent was obtained from each participant. Respondents were interviewed by trained interviewers using structured questionnaire at office of WPDC ensuring their privacy. Information was obtained including background characteristics of respondents, family history of hypertension, smoking status, presence of other chronic diseases, duration of hypertension, mode of diagnosis, number of drugs taken, regularity of taking drugs, and difficulties in complying with treatment.

Regarding the knowledge, subjects were asked about the possibility of total curing of hypertension. They were also asked whether treatment should be stopped if blood

pressure was controlled. Moreover, age and gender susceptibility, causes and complications of hypertension, and if it was possible to prevent the disease were sought. In this regards, respondents answered to the questions till the single response was obtained.

Blood pressure was taken two times, one time before and another after interview. The first measurement was taken after at least 15-minute rest in a sitting position and was followed by another immediately after interview. Interview took about 15-20 minutes. BP was measured manually according to the standard protocol. Readings were based on Korotkoff first and fifth phase sounds. Measurements were done by a medical officer who was assigned for it. The mean values of the 2 separate readings were used as systolic and diastolic blood pressure of the participants.

Working definitions

Compliance was measured by therapeutic outcome method where blood pressure less than 140/90mmHg was considered as the indicator of treatment compliance.

Statistical methods

The data was analyzed using the Statistical Package for Social Sciences (SPSS), version 10. Frequency distributions were used to show the background characteristics of the respondents. The chi-square test was used to test the significance of association between categorical groups. Set 'p' value <0.05 was considered statistically significant.

Ethical consideration

This study was approved by the Institutional Ethical Review Committee, Department of Medical Research (Lower Myanmar).

RESULTS

Characteristics of the respondents

The study population included 105 females and 28 males who were eligible for the study. The overall mean age was 54.67 ± 12.77

years. Majority of the respondents (70.7%) were married while the rest were widowed, divorced or separated. Only 2.3% were single. Overall, no formal, primary, middle and high school and above education were 22.5%, 30.1%, 28.6% and 18.8% respectively. Regarding the occupation, most of the respondents were dependents (65.4%), followed by own business/office staff (22.6%) and unskilled labours (12.0%). The median family income was 50,000 Kyats/month (range, 2,200-150,000 Kyats / month).

The median duration of hypertension was 4 years (Range: 0.5-38 years). Overall mean systolic blood pressure (SBP) and mean diastolic blood pressure (DBP) were 144.18 ± 22.47 mmHg and 91.05 ± 11.57 mmHg respectively. Among the respondents, 47.4% had family history of hypertension and 17.3% had other chronic diseases such as diabetes mellitus, ischaemic heart disease and osteoarthritis. Smoking habit was found in 21.1% of the respondents. Ninety-four respondents (70.7%) reported that hypertension was diagnosed when presenting with symptoms of disease such as headache and dizziness, while in 39 respondents (29.3%) diagnosis was made accidentally.

Treatment compliance

100/133 (75.2%) of respondents reported that they took treatment for hypertension. According to the therapeutic outcome (BP < 140/90 mmHg), 39 patients (29%) were seem to be compliant. The mean SBP was 118.65 ± 11.1 mmHg in the compliance group, while in the non-compliance group it was 152.39 ± 18.11 mmHg. The mean DBP was 76.79 ± 6.69 mmHg in compliance group while it was 95.69 ± 8.22 mmHg in non-compliance group. Compliance was better among subjects aged < 45 years compared with those in older age groups ($p=0.006$). There were no significant differences in compliance rate between gender, education, occupation, marital status and monthly income (Table 1).

As regards treatment compliance, a significantly higher compliance was observed in

Table 1. Effect of demographic variables on compliance rate (BP <140/90 mmHg)

Variables	Total	Compliance		Signifi- cance (p value)
		No.	%	
<i>Age (years)</i>				
<45	33	16	48.5	0.006
45-54	29	10	34.5	
55-64	39	10	25.6	
≥65	32	3	9.4	
<i>Sex</i>				
Male	28	6	21.4	0.36
Female	105	33	31.4	
<i>Marital status</i>				
Single	3	1	33.3	0.31
Married	94	1	33.0	
Divorced/ widow/ widower	36	7	19.4	
<i>Education</i>				
No formal education	30	4	13.3	0.15
Primary school	40	14	35.0	
Middle school	38	14	36.8	
High school & above	25	7	28.0	
<i>Occupation</i>				
Dependent	87	24	27.6	0.82
Unskilled labour	16	5	31.3	
Own business/office	30	10	33.3	
<i>Income(in kyats) (n=126)*</i>				
≥50000	70	21	30.0	0.9
<50000	56	18	28.3	

* Did not include 7 patients, who did not respond willingly

patients who has taken drugs as prescribed compared to those who did not follow their doctor's advice for taking drugs (42.5% Vs 23.7%, $p< 0.038$). Other factors such as duration of having hypertension (Median: 4 years; Range: 0.5-38 years), presence or absence of difficulty with getting treatment and of having chronic disease, numbers of drugs for hypertension, mode of diagnosis of hypertension were not found to be associated with the compliance rate (Table 2). In this study, only a small number (9 patients) went on the regular follow-up.

Knowledge of hypertensive patients on disease

Knowledge of hypertensive patients on disease aspect are presented in Table 3. Almost two-thirds (61.7%) of patients believed that hypertension could have a permanent cure and 60.2% thought that they would stop the medications once control was achieved. Regarding on age and gender susceptibility to hypertension, 46.6% of patients thought that middle-aged persons

Table 2. Effect of treatment variables on compliance rate (BP<140/90 mmHg)

Variables	Total	Compliance		Significance (p value)
		No.	%	
<i>Duration of hypertension</i>				
<4 years	71	21	29.6	0.9
≥4 years	62	18	29.0	
<i>Difficulty of getting treatment</i>				
Yes	53	16	30.2	0.9
No	80	23	28.8	
<i>Having other chronic disease</i>				
Yes	25	8	32.0	0.81
No	108	31	28.7	
<i>Drugs taken as prescribed</i>				
Yes	40	17	42.5	0.038
No	93	22	23.7	
<i>Number of drugs (n=118)*</i>				
One	59	17	28.8	1
Two or more	59	17	28.8	
<i>Mode of presentation</i>				
With symptoms	94	27	28.7	0.84
Accidental finding	39	12	30.8	

*Did not include 15 patients who took either no drug or traditional medicine as an additional drug

were more likely to contract the disease than elderly and younger ones. More than half (54.9%) of the respondents reported that the occurrence of hypertension was more common in females than males. About two-thirds (34.6%) of subjects knew that excessive salt intake is the major risk factor of hypertension, while only 1.5% noticed the role of heredity in causing disease. Although 43.6% of the subjects were unaware of the complications of hypertension, about one-third (31.6%) of the respondents knew that hypertension might lead to cardiovascular complications. Neurological and renal complications were identified by 18.0% and 6.8% of patients respectively.

DISCUSSION

Non-compliance to blood pressure lowering medication is a major reason for poor control of hypertension worldwide. This study revealed that only 29% of patients had compliance with respect to treatment, although 75.2% of respondents had been receiving treatment for hypertension. Compliance rate in our study is low compared to

Table 3. Knowledge of hypertensive patients about the disease

Disease aspect	N0. %	
	N0.	%
<i>Can hypertension be cured?</i>		
Yes	82	61.7
No	38	28.6
Don't know	13	9.8
<i>Can drugs be stopped once control is achieved?</i>		
Yes	80	60.2
No	49	36.8
Don't know	4	3
<i>Which sex is more susceptible to hypertension?</i>		
Males	12	9
Females	73	54.9
Both males and females	30	22.6
Don't know	18	13.5
<i>Which ages are more susceptible to hypertension?</i>		
Elderly	35	26.3
Middle age	62	46.6
Young age	4	3.0
All ages	23	17.3
Don't know	9	6.8
<i>What cause hypertension?</i>		
Excessive salt intake	46	34.6
Stress	29	21.8
Obesity	26	19.5
Heredity	2	1.5
Don't know	30	22.6
<i>What are the complications?</i>		
Cardiovascular	42	31.6
Neurological	24	18
Renal	9	6.8
Don't know	58	43.6

other studies although there are differences in the methods used. Al-Sowielem *et al.* reported the compliance rate in Saudi Arabia as 34.2% [9]. A study from Nigeria showed good compliance with drug treatment was observed in 54.2% of respondents [10].

Our findings did not show statistically significant differences in compliance with sex, marital status, education and monthly income, but relatively young patients (<45 years) had better compliance rate than older ones. It could be explained by the fact that young patients may be more receptive to hypertension related education from mass media sources. Another possible explanation is the missed doses due to forgetfulness among older patients. Treatment factors associated with the compliance were also determined. In this regard, better compliance among respondents who had taken drugs as prescribed was not surprising, but

emphasizing the need for health care personnel to encourage this habit, especially in the areas with poor control of hypertension. The practice of mono-therapy for hypertension did not have a significant impact on the compliance rate in this study. In support of this, a study from Japan also found that medication compliance was related to the frequency and timing of dosing rather than the number of drugs [11].

The relationship between patients' misconception about hypertension and poor compliance has been documented [12]. In this regard, this study showed that considerable proportion of patients had lack of knowledge on taking medication, such as believing in a permanent cure for hypertension and discontinued drugs once control is achieved. Since these misconceptions have a considerable impact on compliance, it is imperative that health care personnel should correct them through health education. Screening for hypertension of all ages and both sexes will be affected if there are misconceptions about age and gender related susceptibilities, as observed in this study. Furthermore, many patients believe that excessive salt intake is an important risk factor for hypertension but are ignorant of other contributing factors such as emotional stress and obesity. Although positive family history of hypertension was slightly high (47.4%) among patients, only 1.5% of patients were acknowledged of the role of heredity in the etiology of the disease. The findings indicate that there is a need for health education to enhance the compliance and knowledge about the disease among the hypertensive patients in the study area.

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Diarrhoeagenic *Escherichia coli* from childhood diarrhoea

*Myat Thidar, *Phyu Win Ei, *Mi Mi Htwe, *Aye Aye Maw & *Wah Wah Aung

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

A cross-sectional study was done to characterize the diarrhoeagenic *Escherichia coli* (DEC), isolated from stool samples of children under 5 years of age with diarrhoea who were admitted to the Paediatric Ward of North Okkalapa General Hospital from January to September, 2007. DEC was isolated from 16 stool samples among 60 samples collected. Characterization of DEC was done by serotyping, toxin assay and polymerase chain reaction (PCR). Serotyping identified 8 enteropathogenic *E. coli* (EPEC), 3 enterotoxigenic *E. coli* (ETEC), and one enteroinvasive *E. coli* (EIEC). Four isolates were not serotypable. The primers used in PCR assay were LTI-O1 and LTI-O2 for ETEC-Labile Toxin; ST-157 and ST-158 for ETEC-Stable Toxin; STX-O1, STX-O2, STX-101 and STX-102 for enterohaemorrhagic *E. coli* (EHEC). PCR assay result showed 3 EHEC and one ETEC. Latex agglutination test was done for verotoxin (VT) and heat-labile toxin (LT) and ELISA test for heat-stable toxin (ST). Toxin assays showed 4 VT, 4 ST and one with both VT and ST. In this study, serology results did not correspond to toxin assay and PCR results indicating that serotyping alone is not sufficient for diagnosis of pathogenic type for DEC.

INTRODUCTION

Diarrhoea caused by *Escherichia coli* infection is one of the major health problems for children in many developing countries. More than one billion episodes of diarrhoea occur every year among children under one year of age causing approximately 2.5 million deaths [1]. Diarrhoeagenic *E.coli* (DEC) are recognized as five major phenotypes: enteropathogenic *E.coli* (EPEC), enterotoxigenic *E.coli* (ETEC), enteroinvasive *E.coli* (EIEC), enterohaemorrhagic *E.coli* (EHEC) and enteroaggregative *E.coli* (EAEC). The most commonly reported DEC were EPEC, ETEC and EHEC [2].

EPEC strains cause diarrhoea mostly in infants which leads to significant morbidity and mortality. These organisms are recognized by serological testing and they belong to a limited group of specific serotypes. Between 1971 and 1978 many investigators

tested classical serotypes of EPEC strains from patients with diarrhoea for their ability to invade epithelial cells and for the presence of heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST) [3]. ETEC strains that cause diarrhoea in human, which is recognized as traveller's diarrhoea, produce heat-labile and/or stable enterotoxins. It also comprises a heterogeneous array of O: K: H serotypes and enterotoxin phenotypes. These strains were found within a small number of O serotypes including O6, O8, O15, O20, O25, O63, O115, O128, O148 and O159 [4].

EHEC are also important pathogens around the world and it is mostly associated with foodborne outbreaks. It produces Shiga toxins (STX1 and STX2). It is associated with O157:H7 serotypes. Identification of DEC requires that these organisms be differentiated from nonpathogenic members of the normal flora. Serogrouping of O antigen is not sufficient to identify a strain as diarrhoeagenic, because it does not correlate,

in most cases, with the presence of virulence factors [5]. Standard methods currently used in the identification of major *E. coli* pathotypes are based on distinct sets of virulence markers such as toxins (heat-labile and heat-stable enterotoxins). Thus identification of DEC strains needs to detect factors that determine the virulence of these organisms.

Objectives

- To identify diarrhoeagenic *E. coli* isolated from stool samples of children under 5 years of age
- To characterize the isolated DEC by serotyping, toxin assay and polymerase chain reaction assays

MATERIALS AND METHODS

A hospital-based, cross-sectional study was carried out to characterize the diarrhoeagenic *Escherichia coli* (DEC) from under five children with acute diarrhoea who attended the Paediatric Ward of North Okkalapa General Hospital (NOGH) between 1st January and 30th September, 2007. Rectal swabs were collected from 64 children (less than 5 years of age) with acute diarrhoea (≥ 3 watery or loose stools within 24 hours for <14 days) [6], admitted to the Paediatric Ward of NOGH within 9 months period. Rectal swabs were transported in Cary-Blair transport media to the Bacteriology Research Division, Department of Medical Research (LM). Culture and biochemical tests were performed to isolate the *E. coli* colonies in accordance with the WHO standard procedure [7]. Rectal swabs in Cary-Blair transport media were inoculated directly onto the MacConkey agar for culture overnight (18 hours) at 37°C. One to three isolates with typical *E. coli* morphology were initially selected and examined biochemically following Cowan and Steel method [8]. Serotyping assay, polymerase chain reaction (PCR) assay, and toxin assays were performed for all *E. coli* isolates.

Serotyping assay

Colonies isolated from Triple Sugar Iron agar (of biochemical set) were tested for

agglutination with 4 polyspecific antisera A, B, C, D. Strains that were agglutinated by the polyspecific antisera were retested by using 32 monospecific O:K antisera (Difco Laboratories, USA; Denka Seiken Co, Japan).

Identification of virulence markers by PCR

E. coli isolates were subjected to PCR assay using primer sets described in Table 1.

Table 1. Primer sets for detection of enterotoxigenic and enterohaemorrhagic *E. coli*

Category	Target	Nucleotide sequence (5'→ 3')	Size of amplified product (bp)
ETEC - LT	LTI-01	CAAGCTTGGAGAGAAGAACCC	203
	LTI-02	TCATCCCGAATTCTGTTATAT	
ETEC - ST	ST-157	TTTTCTTTCTGTATTATCTT	191
	ST-158	ATTACAACACAATTACAGC	
EHEC	STX-01	ATCAGTCGTCACACTCACTGGT	113
	STX-02	CTGCTGTCACAGTGACAAA	
EHEC	STX-101	CAACACTGGATGATCTCAG	349
	STX-102	CCCCCTCAACTGCTAATA	

ETEC = enterotoxigenic *E. coli*; EHEC = enterohaemorrhagic *E. coli*; LT= heat-labile enterotoxin; ST =heat-stable enterotoxin; STX = Shiga toxin; bp = base pair.

Boiled lysates from overnight-grown bacterial colonies were used as PCR templates. The reaction mixtures were run in a thermal cycler (PCR Sprint Thermal Cycler, Thermo Electron corporation) with the following cycling profile: 25 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 60 seconds and primer extension at 72°C for 60 seconds. Amplified products were resolved by 2% agarose gel electrophoresis and visualized under UV transillumination after ethidium bromide staining. DNA templates from positive and negative control strains for virulence markers and minus-template samples were included in each PCR [9].

Toxin assays

E. coli isolates were inoculated into 2 ml of sterile CAYE broth. The culture was incubated with continuous and vigorous shaking at 37°C for overnight (18 hours). After incubation, the culture broth was

centrifuged at 900g for 30 minutes at 4°C and supernatant was used as the test sample. *E. coli* ST EIA (Oxoid) was used for detection of heat-stable *E. coli* enterotoxin, VET-RPLA (Oxoid) for detection of heat-labile *E. coli* enterotoxin and VETC-RPLA (Oxoid) for detection of verotoxins VT1 and VT2 of *E. coli* were performed according to instruction in the test manuals accompanied in each test kit [10, 11, 12].

RESULTS

There are 16 *E. coli* isolates from 60 rectal swabs. Among them, 12 serotypes were identified by serotyping assay. Four isolates were not serotypable with antisera used in this study (Table 2).

Table 2. Result of serotyping assay, PCR and toxin assays for 16 *E. coli* isolates

No.	Lab. ID	Serotyping assay		PCR	Toxin assay
		Serotype	Pathotype		
1	1	O55:K59	EPEC	-	-
2	2	Non-typing		EHEC	-
3	5	O27:K+	ETEC	EHEC	-
4	8	O1:K51	EPEC	-	-
5	9	O157:K+	EPEC	-	-
6	13	O8:K40	ETEC	-	-
7	14	O8:K25	ETEC	EHEC	VT
8	18	O1:K51	EPEC	-	ST
9	16	O125:K70	EPEC	ETEC	ST
10	21	O127:K63	EPEC	-	ST
11	25	Non-typing		-	VT
12	19	Non-typing		-	VT
13	31	O112:K66	EIEC	-	ST
14	36	O44:K74	EPEC	-	VT, ST
15	38	O125:K70	EPEC	-	-
16	48	Non-typing		-	VT

According to toxin assays, there were 4 isolates positive for heat-stable enterotoxin (ST) and 4 isolates positive for verotoxin (both VT1 and VT2). One isolate was positive for both ST and VT. Two pathotypes were identified in 4 isolates on the basis of virulence marker profile by PCR assay. There were three isolates which showed pathotype EHEC (positive for STX-01, STX-02, STX-101, STX-102) and only one isolate was ETEC pathotype (positive

for ST-157, ST-158). There was no isolate positive for LTI-01, LTI-02 marker in this assay. The detected heat-stable toxin gene and verotoxin gene of *E. coli* by PCR in this study are shown in Fig. 1 and 2.

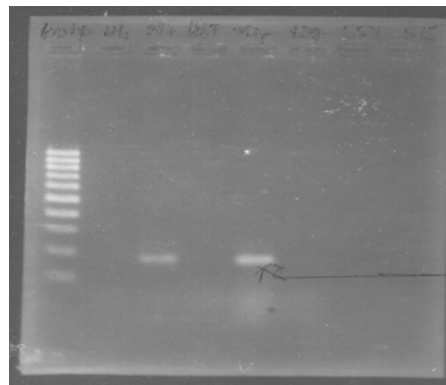


Fig. 1. Detection of heat-stable toxin gene of *E. coli* by PCR (191 bp)

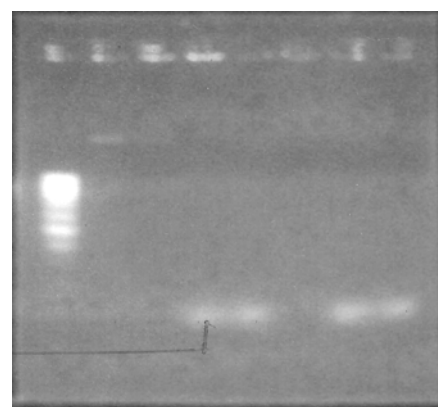


Fig. 2. Detection of verotoxin gene of *E. coli* by PCR (113 bp)

In this study, pathotypes identified according to serogroups did not correspond with the PCR identified pathotypes and with toxin assays. There were two isolates, (Lab ID 14 and 16 in Table 2) which were EHEC and ETEC according to PCR assay and these two isolates showed corresponding results in toxin assays as VT and ST respectively.

DISCUSSION

Diarrhoea remains one of the main causes of morbidity and mortality in children and a large proportion is caused by diarrhoeagenic *E. coli*. Many of the virulence determinants

have been determined and some DEC produce toxins. The virulence factors of some DEC have yet to be fully determined and in the meantime they remain large and emerging problems without the availability of effective vaccine [13].

O, H and K antigens of *E. coli* are used to classify the isolates for epidemiologic purposes. Specific antigenic serogroups are also associated with greater virulence [14]. In this study, 12 serotypes were identified among 16 isolates from 60 rectal swabs collected during study period. These serotypes were identified as 8 EPEC, 3 ETEC and one EIEC according to manufacturer of antisera.

The results of toxin assays showed that 9 isolates produced toxins, among them 4 produced heat-stable toxin, 4 produced verotoxin and one produced both verotoxin and ST toxin. None of the isolates produced heat-labile toxin. When the serogroup defined pathotypes were compared with toxin assays, there was one ETEC serotype (O8:K25) that produced verotoxin and three EPEC (O1:K51, O125:K70, O127:K63) which produced heat-stable toxin and one EPEC (O44:K74) that produced verotoxin and heat-stable toxin. One EIEC (O112:K66) produced heat-stable toxin and three non-serotypable isolates produced verotoxin. This finding shows pathotypes determined by serotyping assays are not compatible with toxin assay results. Toxin production is not associated with specific serogroups and tissue cultures, animal model assays or *in vitro* assays must be performed if toxigenic strains are to be detected. Nucleic acid probes have also been used to detect the toxin genes [14]. In this study nucleic acid assay was performed by using primers targeting virulent genes for ETEC and EHEC. The primers for EPEC and EIEC were not included in this study. Results of PCR assay showed three EHEC and one ETEC, which were different from results obtained by serotyping.

Diarrhoeagenic *E. coli* isolates from 217 children (aged under five years) who had

been admitted to Yangon Children's Hospital in Myanmar between 1999 and 2003 were studied by Eizo Takahashi and group [15]. In that study, 47 isolates possessed virulence genes characteristics of diarrhoeagenic *E. coli* among which 30 were EAEC, 12 were EPEC and 5 were ETEC.

Serotyping has classically been used as the standard assay to detect strains of diarrhoeagenic *E. coli*. Although serotyping is conventional, most serogroups of diarrhoeagenic *E. coli* are not restricted to only one pathotype. Also the serogroups that had been considered to represent diarrhoeagenic *E. coli* have also been considered to represent among non-diarrhoeagenic *E. coli*. For these reasons, serotyping is not considered to be a reliable method for detection of diarrhoeagenic *E. coli* [16, 17].

This study also shows that serotyping assay alone is not sufficient to determine the pathogenic type of *E. coli* and the role of PCR assay and toxin assays are more important in determining the pathogenic type of *E. coli*. In future, more sensitive and specific tests using nucleic acid probes should be established to characterize the diarrhoeagenic *E. coli* that causes childhood diarrhoea in Myanmar.

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Perinatal transmission of Group B Streptococcal infection

**Cho Cho Thet, *Moe Moe Tun,*Thein Tun, *Khin Lin,
*Maung Maung, **Mar Lar Win, **Aye Aye Hlaing &***Kyi Kyi Myint*

**Department of Medical Research (Upper Myanmar)*

***University of Medicine (Mandalay)*

****Obstetrics and Gynaecology Department
300 bedded Hospital (Mandalay)*

Group B Streptococcus (GBS) is a leading cause of invasive bacterial infection in newborns and also affects pregnant women. The objective of this study was to determine the vertical transmission of GBS infection from mothers to fetus. It was hospital-based cross-sectional descriptive study. One hundred and fifty pairs of pregnant women and their neonates were studied in Mandalay 300 bedded hospital between May, 2006 and April, 2007. Swabs from both vagina and rectum from mothers and ear, nose and umbilicus from neonates were collected and cultured on selective media. Risk factors were investigated by chart review and hygienic status of mothers was assessed by investigators. The overall maternal GBS colonization rate was 16% and perinatal transmission rate was 75% (n =18/24). Significant vertical transmission was observed from colonized mothers to their infants (OR = 91.5) (p < 0.001). The association between maternal GBS colonization and maternal conditions such as prolong labour, fever and premature rupture of membrane (PROM) was observed. Erythromycin and tetracycline were the most sensitive drugs on GBS. Ampicillin was effective in vaginal site colonization only. Penicillin was effective in ear colonization of the neonates only. This finding would be helpful to health-care providers in management of GBS infection among mothers and infants.

INTRODUCTION

Group B streptococcus (GBS), is the most common cause of life-threatening infections in newborns, especially sepsis and meningitis. GBS is a frequent cause of pneumonia and is more common than other, better known, newborn problems such as rubella, congenital syphilis, and spina-bifida. Aggressive intravenous antibiotic therapy successfully treats most babies who develop a GBS infection, but even with the best medical care 10-20% of these babies die and a few suffer long-term problems, most usually those who developed GBS meningitis. The prevalence of GBS colonization in various populations has ranged

from less than 5% to more than 40%. One of the negligible signs for GBS infection is lack of examination of maternal GBS colonization, which are commensals for mothers and colonized women have no symptoms of disease. Approximately 8000 babies in the United States get GBS disease each year and 5-15 % of these babies die. GBS is a common type of the streptococcus bacterium. Approximately a quarter of childbearing age women carry it in their vagina. These women have potential for transmitting this organism to their newborn infants vertically or rarely, by hematogenous dissemination. The prevalence of GBS in pregnant women varies from place to place as reported by several workers the world over. Rates of

GBS colonization remain unpredictable and vary geographically, while rates of GBS disease are less often reported. GBS remains a leading cause of sepsis and pneumonia in the newborns despite the implementation of universal prenatal screening and intrapartum antibiotic prophylaxis in pregnant women [1]. The most important problem in the prevention programme is the identification of the cases to treat, since it is not possible to give antibiotics to all women. Infection because of GBS is acquired through fatal aspiration of infected amniotic fluid or during passage through the vaginal canal [2]. More epidemiological studies and pathogen transmission, characterization and formulation of clinical management are urgently needed in both mothers and their neonates.

Currently, neonates born to women with unknown GBS status at delivery are unclear. So this study was done to provide the baseline data on GBS occurrence, transmission and their risk factors between mother and fetus.

MATERIALS AND METHODS

All the 150 pairs of enrolled neonates and their mothers underwent collection of specimens from surface sites for culture and sensitivity patterns from May, 2006 to April, 2007. All the specimens were collected by the investigators. Maternal personal hygienic status was identified by investigators. Good personal hygiene of mothers defined as they had neat and tidy hair condition, healthy look skin and short and clean nails. Poor personal hygienic conditions were in contrast to above criteria. Maternal complication status was stated in this study that the mothers had at least one or more of the following conditions such as maternal fever, prolong labour and premature rupture of membrane before delivery in the hospital.

Two vaginal and two rectal swabs were collected from each mother before no anti-

septic application of the perineum or vulva. A total of three samples were collected from each newborn infant immediately after birth viz. external ear, nasal and umbilical swabs. These samples were immediately immersed in Todd-Hewitt broth (with colistin 10mcg/ml and nalidixic acid 15 mcg/ml) and sent to the Microbiology Department for staining and culture and sensitivity. These special broth media were incubated overnight at 35 to 37°C for 18-24 hours. And then they were sub-cultured onto Granada medium and incubated again at 35 to 37°C for 18-24 hours. Gram stains were also applied on these swabs and colonial growth from culture. The isolates were tested for antibiotic sensitivity by disc diffusion method of Kirby-Bauer by employing drugs on 5% sheep blood agar.

RESULTS

Socio-demography of study population

In our study, the number of births contributed by 20-29 years and 30-39 years age group were 67/150 (44.7%) and 63/150 (42%), respectively. Among these populations, most of the mothers were educated up to high school level (28.67%), followed by primary school level (28%), middle school (14%), illiterates (20%) and highly educated (9.33%). Most of the mothers were dependants (69.33%) and 19.33% of mothers could work on their own occupational status. Government staff and private workers were 6.0% and 5.33 %, respectively. Good personal hygienic status and poor personal hygienic status were 86.7% vs. 13.3%. In our study 2% of mothers had feverish condition during the study period. The temperature was >38° C. But almost all the mothers in this study were in state of well-being. In this study period, only 4 of 150 (2.7%) mothers present prolong labour. But all the rest of the mothers in our study came to the hospital with intact membrane. Only 6% (9/150) of pregnant women presented with premature rupture of membrane.

Maternal GBS colonization status was 16% in this tested population. In this study, neonatal GBS colonization rate was 14.7% (22 out of 150) and 85.3% of the neonates have no colonial growth. Overall nasal colonization rate in this study was 12% (18 out of 150); ear colonization rate and umbilical colonization rate were 10% and 6.67%, respectively. Both ear, nasal and umbilical regions were commonly found in these studied area (36.6%), second most common site was nasal region (22.7%) and the second last was ear and nasal region (18.1%). Umbilical region was the lowest colonization site (4.5 %).

In this study, erythromycin and tetracycline were the most sensitive drugs of this study population. Their sensitivity rates were more than 70% except to rectal site for erythromycin and nasal site for tetracycline. In vaginal site, ampicillin was effective in treatment for this organism only and penicillin could also be used in treatment of GBS colonization of ear site.

Association between GBS infection of mothers and neonates

Mothers with GBS colonization status during labour were 91.5 times more likely to infect newborns with streptococci (OR 91.5: 95% CI 20.05-476.81, $p < 0.001$). Perinatal transmission rate was 75% (18 out of 24). Twenty-five percent (6 out of 24) of mothers could not give GBS transmission to their newborn infants in this study. But 18.1% ($n=4/22$) of newborns could not get vertical transmission of GBS from their mothers even though their mothers colonized with GBS. Maternal age, educational status and personal hygienic condition of mothers and vertical transmission are shown in Table 1, 2 & 3. Maternal complications (such as PROM and fever $>100.4^{\circ}\text{F}$) were 2 times more likely to get infected newborn with streptococcal infection (OR: 2.09 95% CI 0.4-9.7, $P=0.2479$). Data demonstrating the relationship between intrapartum colonization of vagina and infant GBS infection

were quite compelling (OR: 20.05 95% CI 1.68-537.7, $p=0.0100$).

The risk factors

Table 1. Age of mothers and neonatal GBS colonization status

Age	Neonatal GBS		Total
	Positive	Negative	
> or = 35 year	6	31	37
< 35 year	16	97	113
Total	22	128	150

(OR: 1.17: 95% CI: 0.37-3.6, $p=0.4713$)

Table 2. Association between maternal education and neonatal GBS colonization

Education	Neonatal GBS		Total
	Positive	Negative	
Middle school and above	18	102	120
Primary school and Illiterate	4	26	30
Total	22	128	150

(OR: 1.14: 95% CI: 0.32-4.45: $p=0.5395$)

Table 3. Personal hygienic condition and neonatal GBS colonization

Personal hygiene	Neonatal GBS		Total
	Positive	Negative	
Positive	5	15	20
Negative	17	113	130
Total	22	128	150

(OR: 2.21: 95% CI: 0.6-7.78: $p=0.1438$)

DISCUSSION

Group B *Streptococcus* (GBS) is an important cause of infection in pregnant women and their newborns; however, there had been a few studies in Myanmar. The association between the presence of the organism in the birth canal and invasive neonatal disease was recognized soon after the emergence of GBS as a major cause of neonatal infections, and hence the epidemiology of maternal colonization has been investigated extensively. This study revealed that the overall maternal GBS prevalence rate is 16% which is within the range of 5%-25% as documented in different parts of the world. But the occurrence rate of colonization in Mandalay is relatively high when comparing to reports from Yangon,

Myanmar (7.1%) [3]. According to these studies, GBS colonization rates remain variable according to geography. One study reported that colonization rates in the pregnant women ranged from 2% to 35% [4].

GBS recovery percentage from vaginal site and rectal site was 25% by culture assays in this study. Twelve out of twenty-four (50%) of women colonized in both rectal and vaginal region. Schuchat A & Wenger JD [5] stated that these bacteria asymptotically colonize the vaginal or rectal areas of 10 to 30% of pregnant women and 40% to 70% in neonates born to colonized mothers. The prevalence of GBS colonization by culture in another study was 17% which is concordant with the reported rates of maternal GBS carriage and vertical transmission [6]. The other six published studies from Latin America provide evidence of geographical variation in maternal GBS colonization. These women with GBS colonization are considered candidates for intrapartum anti-biotic prophylaxis to prevent further transmission to neonates [7].

Between 30 and 70% of infants born to mothers with GBS colonization also become colonized at rectal, umbilical or oral sites [8]. The most three common sites for GBS colonization in infants are ear, nasal and umbilical region in our study. But overall incidence of each sites of neonates are nasal area (12%), ear site (10%) and umbilical area is only 6.67 %.

In our study, newborn infants were definitely to be infected with GBS while their mothers had GBS colonization (OR: 91.5; 95% CI: 20.05-476.81, $P < 0.001$). Some of them would escape from vertical transmission even though the chance of transmission was high. One in 4 of colonized mothers could not give perinatal transmission but 1 in 5 neonates could get GBS colonization even their mothers did not colonize with Group B *Streptococcus*. Vaginal colonization with GBS is essentially a prerequisite for both early colonization of newborn infant and early onset GBS [9, 10]. Failure of transmission from GBS

colonized mothers to fetus may depend on density of maternal colonization. Many comparative studies showed that infants whose mothers have heavy genitourinary GBS colonization are more likely to become colonized [11] and the risk for GBS infection is much greater in heavily colonized neonates [12]. Failure to culture GBS even with ideal sampling and culture techniques may also be caused by maternal factors. The use of oral antibiotics or a variety of feminine hygiene products (including douches, vaginal candidiasis medication, and inert lubricants) before specimen collection may inhibit GBS growth in culture and contribute to false-negative results [13].

Our study also revealed that all the GBS isolated from both mothers and fetus are mostly sensitive to erythromycin and tetracycline assuming that sensitivity percentages over 70% act as a sensitive drug for usage and the rest of the tested drugs such as penicillin, ampicillin, gentamycin and kanamycin have less sensitivity rate because their sensitivity percentages are less than 70%. Karen MP *et al.* [14] also reported that 20% were resistant to erythromycin and clindamycin. But in the report of Simoes J *et al.* [15], 17% of isolates were resistant to ampicillin and 15% showed penicillin resistance. Widespread use of antimicrobials is known to increase the risk for emergence of antimicrobial-resistant organisms and so surveillance is indicated to determine the potential for these phenomena.

The perinatal transmission rates also showed association between carriage of GBS among neonates and personal hygienic status of mothers and their education status. In this study, unhygienic condition of mothers can give 2 times likely to get GBS colonization in neonates (OR: 2.21; 95% CI: 0.71-6.88; $P=0.1438$). Ethnicity, maternal age and parity, marital status, education, smoking and frequent intercourse with multiple partners also may influence the prevalence of colonization [16]. Prolong labour was presented only in 4 of 150 GBS

infected infants and it was significantly associated with neonatal GBS colonization (OR: 20.05; 95% CI: 1.68-537.75, $p=.2479$). Prolong rupture of the membranes for >18 to >20 hours before delivery substantially increase the risk of neonatal GBS disease [17, 18]. Intrapartum temperature of >38°C is associated with vertical transmission of neonates in this study. One study stated that intrapartum fever of >38°C is associated with an increased risk of neonatal GBS infection [19]. This study was similar to the report of Cimolai and Roscoe [20] where GBS bacteraemia in infants born to mothers with a temperature of >37.5°C. All the GBS positive infants were asymptomatic in this study. The high incidence of GBS neonatal transmission in our survey may be related to the high maternal colonization rate with GBS in this area.

In conclusion, mothers with GBS bacteriuria during pregnancy can give perinatal transmission to their infants. Until effective GBS vaccines are available for clinical use, rapid and accurate diagnostic screening of women when they present for delivery may be the most effective approach to additional prevention of neonatal GBS disease in the current era. For now, culture of specimens from the rectum and lower vagina remain the gold standard for detecting GBS colonization in pregnant women [21]. The antibiotic selected for intrapartum chemoprophylaxis should be guided by the organism's antibiotic sensitivity pattern. Group B streptococcus is the most common cause of life-threatening infections in newborns especially umbilical sepsis, meningitis, pneumonia which can be occurred acutely and it can cause long-term complication such as vision or hearing disability.

Proper treatment with effective antibiotic can cure the disease. In our study, we recommend that erythromycin and tetracycline are the best choice for treatment and so outcome of study will provide the prevention and control of GBS diseases in Upper Myanmar.

Recommendations

1. Maternal health knowledge for personal hygiene and education should be scaled up to prevent transmission of infection to the infants.
2. Measures to reduce prolong labour should be encouraged to lessen perinatal GBS transmission.
3. Erythromycin should be provided as appropriate antibiotic prophylaxis for pregnant women having prolong labour.

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Quality assured of the recombinant hepatitis B vaccine produced at the GMP standard Hepatitis B Vaccine Plant, DMR (LM) (2003-2006)

Win Aung, **Khin May Oo, *Moh Moh Htun, ****Zaw Myint, *****Win Maw Tun, *****Khin Khin Aye & *****Khin Pyone Kyi*

**Blood Programming Division*

***Experimental Medicine Research Division*

****Pathology Research Division*

*****Biochemistry Research Division*

******Bacteriology Research Division*

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

For effective control of hepatitis B viral infection in Myanmar, the GMP standard Hepatitis B (HB) Vaccine Plant was established in 2003. In this plant, the recombinant hepatitis B vaccine has been produced in compliance with the WHO GMP recommendations since 2004. The major purpose of applying for the WHO GMP is to achieve a high quality product. The quality of the product is individually or collectively influenced by a variety of factors such as men (personnel), machines, materials and methods used in manufacturing processes. In this descriptive study, quality assurance of the recombinant hepatitis B vaccine was assessed by monitoring the following systems in the plant; documentation, training, audit, preventive maintenance, calibration, validation, clean environment, raw material and product control, in-process control, change control and investigation of deviation and customer's complaint, and annual product review. It was found that the location, layout design and facilities of the plant building and premises were in accordance with WHO GMP recommendations. The standard organization set-up and effective quality management system had already been established. Production processes and quality control testing were regularly carried out by qualified and trained staff under strict aseptic technique in well controlled clean rooms by using established batch processing records and SOPs, standard materials and chemicals, and well calibrated and validated equipment. The starting raw materials, intermediate and finished products passed the specifications of quality control tests recommended by WHO. The results of the phase I and phase III clinical trials carried out on 17 adult volunteers and 134 neonates (target population) respectively showed the safety and 100 % immunogenicity of the vaccine. Therefore, recombinant HB vaccine, locally produced at the HB vaccine plant, Department of Medical Research (Lower Myanmar) has been proven to be a high quality product, and found to be totally safe, immunogenic and capable of protecting against HB viral infection. It is also considered to be the WHO GMP acceptable quality product for use in global immunization programme.

INTRODUCTION

Hepatitis B (HB) viral infection is an important health problem worldwide because the infection can cause significant liver diseases. Myanmar is hyperendemic for HB

viral infection as research studies have shown that the HB carrier rate is 10-12 % and infection rate, 35-65 % [1, 2]. Recombinant hepatitis B(HB) vaccines using recombinant DNA technology entered the market and were registered at US FDA in 1986 [3]. In

Myanmar, the contract for the Hepatitis B Vaccine Plant Project under EDCF loan of US\$ 12.6 million was signed in 2001 between (DMR, LM) and Samsung /CJ Corporation, Republic of Korea, for the development of yeast-derived recombinant hepatitis B vaccine in Myanmar. According to the contract, the supplier has to build a WHO Good Manufacturing Practice (GMP) standard HB Vaccine Plant scheduled to produce 5 million doses of yeast (*Hansenula polymorpha*) derived recombinant hepatitis B vaccine annually. The construction of the plant and test production of vaccines was completed in April 2003 and April 2004 respectively. All steps of manufacturing and quality control testing were carried out in accordance with WHO GMP recommendations. Clinical trials on adult volunteers and neonates were successfully carried out in 2005 and 2006 respectively, showing safety and immunogenicity in vaccine recipients. The major purpose of applying for the WHO GMP in this Plant is to achieve and ensure the high quality of the product. The quality of the product is individually or collectively influenced by four basic elements of GMP; men (personnel), machines, materials and methods used in the vaccine plant. These elements are controlled by the established quality management in the Plant. In this study, quality management system applied in the GMP standard DMR HB Vaccine Plant was systematically assessed with the aim of ensuring that the recombinant HB vaccine produced at the Plant is a high quality product acceptable by WHO for use in the global immunization programme.

MATERIALS AND METHODS

This is a descriptive study carried out at the GMP standard HB Vaccine Plant where recombinant HB vaccines are produced and controlled to the quality standard recommended by WHO GMP. The quality of vaccine is ensured by controlling the quality management system, starting from the receipt of raw materials, through in-process products

to the release of finished products. In this study, quality assurance of the recombinant HB vaccine was assessed by monitoring the following systems in the Plant; documentation, training, audit, preventive maintenance, calibration, validation, clean environment, raw material and product control, in-process control, change control, investigation of deviation/customer's complaint and annual product review.

RESULTS

The DMR (LM) HB Vaccine Plant is located at the corner of No 7 Main Road and Kha Yay Pin Road, Hlegu Township, Yangon Division, 40 kilometers away from Yangon downtown area. Figure1. illustrates the layout design of the main building in the HB Vaccine Plant with having flow patterns of personnel, material, process, product, waste, and air pressure.

Educational qualification and specialties of the 20 scientists working in the Plant are shown in Table 1. Thirteen scientists (65%) hold post graduate degrees and 18 scientists (90%) had vaccine-related training abroad.

Table 1. Educational qualification and specialties of 20 scientists working in the Plant

Qualification / Specialities	No. of Scientists (%) n=20
Post graduate degree	13 (65%)
PhD	4 (20%)
MMed Sc/ MSc	9 (45%)
MBBS	12 (60%)
BSc	9 (45%)
Diploma	1 (20%)
Training abroad	18 (90%)
Service ≥ 15 years	15 (75%)
Medical Doctor	9 (45%)
Microbiologist	6 (30%)
Biochemist	2 (10%)
Molecular Biologist	1 (5%)
Pathologist	1 (5%)
Biologist	3 (15%)
Chemist	3 (15%)
Economist	1 (5%)
Engineer	4 (20 %)



Fig.1. Lay-out design of the main building in the HB Vaccine Plant, Hlegu Township

Table 2 shows the in-process control results of intermediate products and purified bulks of different production lots showing the consistency of the production processes.

Table 2. Consistency of in-process products and purified bulks in production process

	Lot no.			
OD of seed culture at 24 hours	R.02	R.03	R.04	R.05
Weight of cell cake after 120 hours	15.9	14.93	15.59	15.25
Fermentation (gm/ litre)				
Protein concentration of purified bulk (mg/ml)	137.6	140.8	138.8	130.0
	0.75	0.68	0.65	0.58

Table 3 depicts the results of the quality control testing of water for injection (WFI) and waste water eluted from waste water treatment system in the Plant. The results of WFI and waste water testing were found be within the expected specifications and acceptable for pharmaceutical use and safety to the environment respectively. Results of the Quality Control testing of the

in-process and finished products are summarized in Table 4, passing the respective specifications recommended by WHO.

Table 3. Quality control testing of water for injection (WFI) and waste water

Test items	Specifications/ Results	Interval of testing
WFI		
pH	5 – 7	Daily
Conductivity	≤ 1.3µsm/cm at 25°C	Daily
Total organic carbon(TOC)	≤ 500 PPB (µg/L)	Daily
Endotoxin	≤ 0.25 EU/ml	Daily
Sterility(Microbial count)	< 1 CFU/10ml	Daily
Waste water		
Temperature	< 40°C	Daily
pH	6.5-7.5	Daily
Biochemical oxygen demand	< 40 mg/L	Every 4 month
Chemical oxygen demand	< 30 mg/L	/
Dissolved solids	< 15 mg/L	/
Suspended solids	< 1000 mg/L	/

Table 5 summarizes the findings of environmental monitoring performed in clean rooms of production areas. The results

measured were found to be within the specifications as recommended by WHO GMP.

DISCUSSION

The WHO defines Quality Assurance (QA) as a wide- ranging concept covering all matters that individually or collectively influence the quality of a product. It is the totality of the arrangement made with the object of ensuring that pharmaceutical products are of quality required for their intended use.

Table 4. Quality control testing of the in-process and finished products of recombinant HB vaccines

No.	Test items	Specification / results
<i>Purified Bulk / Final Bulk</i>		
1	Protein content	The measured value
2	Polysaccharide content	Not more than 10 µg / 100 µg of Protein
3	Lipid content	Not more than 10 µg/100 µg of Protein
4	Pyrogenicity	The summed increase temp is not more than 1.3°C
5	Sterility	No evidence of the presence of bacteria or fungi
6	Antigen content	The measured value
7	Purity	
	Tween 20 content	Not more than 50 µg/100 µg of Protein
	Bromide contents	Not more than 50 µg/100 µg of Protein
8	Host derived DNA	Not more than 100 ng/ dose
9	Identification(SDS PAGE)	Identified at about 24 KDa
<i>Finished Product</i>		
1	Characteristics	Whites turbid solution
2	pH	5.9-6.9
3	Sterility	No evidence of the presence of bacteria and fungi
4	Abnormal toxicity	At least 80% of mice survive the test
5	Thimerosal content	0.007-0.012 w/v %
6	Protein contents	Not more than 40 µg/ml
7	Aluminum content	Not more than 1.25 mg/ml
8	Potency	The lower value of 95% confidence limit is not less than 1.0
9	Filled volume	Average and individual as specified
10	Leak test	No leak is detected
11	Foreign matter	No foreign matter is detected
12	Test for glass container	Not more than 2 ml
13	Pyrogenicity	The summed increase temp is not more than 1.3°C

GMP is that part of QA which ensures that products are consistently produced and

controlled to the quality standards appropriate to their intended use and as required by the marketing authorization. The aim of GMP is to build quality into the product. QA therefore incorporates GMP and other factors such as those of Good Laboratory Practice (GLP) and Good Clinical Practice (GCP). The manufacture of biological products are undertaken in accordance with the basic principles of GMP [4, 5, 6, 7, 8]. In this study, regarding the location and design of the building, it is acceptable for GMP since the plant is not near to public housing, and operating rooms were used with materials of highest standard so that cleanliness can be easily performed and maintained.

Table 5. Environmental monitoring of clean rooms in production areas of the Plant

Types of clean room	Specifications/ Results		
	Class 100*	Class 10,000	Class 100,000
<i>Test items</i>			
Temperature	20°- 22°C	20°- 22°C	20°- 22°C
Humidity	50 ± 5 %	50 ± 15 %	50 ± 20 %
Pressured differential	1.2 -1.5 mm water	1.2 - 1.5 mm water	ND
Air velocity	0.3–0.45 m/sec	ND	ND
Air changes	300 –400 /hr	30 – 40 / hr	20 – 30 / hr
Air direction	Unidirectional & parallel	ND	ND
Lighting	100–150 foot candles	100	100
Noise level	< 60 decibel	< 60 decibel	< 60 decibel
Particle counts (of ≥ 0.5µm)	< 100/ cuft	< 10,000/ cuft	< 100,000/cuft
Microbial counts	< 1 CFU	< 5 CFU	< 50 CFU

* Environmental monitoring was carried out daily during production period and once a week at rest .
ND = Not defined

Positive pressure rooms were used to process sterile products and negative pressure was used in septic rooms where infectious materials were handled. Heating, ventilation, air conditioning (HVAC) and lighting were designed to maintain a satisfactory temperature and relative humidity to minimize contamination and to take into account the comfort of the personnel working in protective clothing.

Regarding the organizational structure and the personnel, there were 3 separated divisions under the plant Director ; Production, Quality Control and Quality Assurance which are independent of each other in accordance with WHO GMP recommendation.. There was also an adequate number i.e a total of 89 scientists and technicians who are aware of the principles of GMP. They are highly qualified with necessary training and practical experience. Their specific duties are recorded in written description and there are no gaps or unexplained overlaps in the responsibilities. During the year under study, 24 lectures on GMP and related topics were given regularly followed by evaluation tests at the Plant. Yearly routine medical check-up of all staff were also performed for detection of infectious diseases.

Daily cleaning of machines and equipment is done by operators at the Plant. Maintenance of a total of 261 equipment and machines is carried out by engineers from the Plant and by the Europ. Continents Co., Myanmar every 6 months. Calibration of 1020 gauges and displays is carried out by engineers from SEOHO Corporation, Republic of Korea once a year. Validation of 39 major equipment, processes, tests and systems including HVAC, water treatment system, Media fill test is performed by QA personnel from the Plant once a year. Equipment used for validation work is scheduled to be recalibrated once a year in the international standard substitute for calibration. Registries of all machines and equipment are also kept systematically in the documentation room as recommended by WHO GMP. Documentation is an essential part of QA system and is the key for operating the Plant in compliance with GMP requirements. No work procedure or tests are performed without established standard operating procedures (SOPs) and Batch Processing Records (BPRs) at the Plant. In addition, in-process quality control testing at every step of the vaccine production process starting from raw materials , through intermediate products to

finished products are carried out to meet the specifications recommended by WHO [9]. The consistency of the results of production lots were observed in this study [10]. During the years under study, 249 SOPs, 12 BPRs, 16 certificates of analysis (COA), and considerable numbers of essential protocols, reports, results and records were prepared, checked and approved. These documents were systematically kept in the documentation room with limited access. A total of 6 internal GMP audit and 2 external GMP audit were carried out by QA personnel of the Plant and authorized persons of Myanmar FDA respectively during the study period. Any change regarding men (personnel), machines, materials and methods used in the plant were strictly controlled and recorded to prevent undesirable effects on product quality. Any deviation from normal processes or test results occurring during the vaccine manufacturing and testing procedures, were thoroughly investigated by QA personnel. The products were recommended to be passed or failed according to the findings. The plant also has an established SOP to investigate customers' complaint, followed by retesting of retention samples, report and product recall if and when necessary. Routine annual product reviews are carried out at the end of each year for documenting any changes and deviation, and for improvement of product quality and yields. The results of the phase I and phase III clinical trials carried out on 17 adult volunteers and 134 neonates (target population) respectively showed total safety and 100 % immunogenicity [11, 12].

Regarding the materials, all chemicals, reagents, buffers and glass vials used at the Vaccine Plant are analytical grade and purchased only from qualified and reputed suppliers. Before use in production process, all incoming materials are serially processed on receipt, lot-in, quarantine, sampling, testing followed by release or rejection. Finished products (vaccine vials) issued from the production division are also subjected to the process of quarantine,

sampling, testing, submission to FDA for lot release followed by ware-housing and distribution. In this study, it was found that there was a well established quality management system in the Plant, systematically and effectively conducted by the Quality Assurance team, controlling men, machines, materials and methods involved in the whole manufacturing process starting from the receipt of raw materials to the release of finished products (vaccines) to ensure high quality product. The production processes and quality control testing in this Plant are consistently carried out in well controlled clean rooms by qualified and trained personnel under strict aseptic technique, using established SOPs, BPRs, standard chemical and tested materials, and yearly calibrated and validated equipment appropriate to their specifications recommended by WHO GMP guidelines. The vaccine on clinical trial has been found to be totally safe, immunogenic and capable of protecting against HB viral infection. Therefore, recombinant HB vaccine locally produced at the HB Vaccine Plant, Department of Medical Research (LM) is recommended to be a high quality product, and it is also considered to be the WHO GMP acceptable quality product for use in the global immunization programme.

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**Analysis of hepatitis C virus isolates from Tamu Township
and identification of a new subtype of genotype 6**

Aye Aye Lwin, *Khin May Oo, **Myo Khin, *Ne Win, *Yi Yi Kyaw,
****Toshiyuki Shinji, ****Norio Koide & ****Shigeru Okada*

*Experimental Medicine Research Division, DMR (Lower Myanmar)

**Department of Medical Research (Central Myanmar)

***National Health Laboratory, Department of Health (Myanmar)

****Okayama University Graduate School of Medicine, Japan

Genotyping of hepatitis C virus (HCV) is important for designing therapeutic strategies and there is little information on the distribution of HCV genotypes in Myanmar. To determine the distribution of genotype/ subtype of HCV isolates from Tamu Township, a total of 501 blood samples were collected and tested for anti-HCV antibody by using the Ortho HCV Ab-PA test II kit. Genotyping was carried out by using reverse-transcriptase polymerase chain reaction (RT-PCR), direct DNA-sequencing and phylogenetic analysis of the partial core sequences. Sixty-four (12.8%) samples were anti-HCV positive and 59 (92.1%) HCV isolates could be genotyped. Genetic characterization of viral sequences indicated that there are three major genotypes (6, 3 & 1) and six minor subtypes as 1a (1.7%), 1b (15.3%), 3b (25.4%), 6m (3.4%), 6n (52.5%) and a new 6 subtype (1.7%). This study showed that HCV genotype distribution in Tamu Township has a distinct geographic variation from other South-East Asia countries in terms of the existence of the new genotype 6 subtype.

INTRODUCTION

Hepatitis C virus is a unique, enveloped, positive, single stranded RNA hepacivirus of the Flaviviridae family. The RNA genome is approximately 9.6kb in length and encodes a single, large polyprotein that is post-translationally cleaved into multiple structural (core, E1 and E2) and non-structural (NS2–NS5) peptides [1]. Currently, all known HCV isolates have been divided into six phylogenetically distinct groups, referred to as clades, and more than 70 subtypes based upon nucleotide sequence and phylogenetic analysis [2]. HCV infection is a major health problem throughout the world. Collective estimates indicate over 175 million people are infected with HCV worldwide with up to 4 million new infections each year [3]. Infection with HCV is the most common cause of progressive liver disease, including

cirrhosis and hepatocellular carcinoma (HCC) [4].

Three general approaches have been employed to characterize viruses and have led to the grouping of viral isolates [1]. The first approach, serotyping, examines the pattern of viral neutralization by antibodies. Another approach, phenotyping, assesses differences in disease patterns, cytopathology or response to antiviral agents when suitable model systems are available. Finally, investigators may look for differences in genetic sequences of viral genes or complete genomes to determine homology and detect variability that may alter viral replication and response to antiviral therapies. This last approach is referred to as genotyping. For some viruses, including HCV, there are no available cell culture systems or appropriate small animal models, making the characterization of these

viruses by serotype or phenotype very difficult. Therefore, genotypic classification and description of viruses has come to prominence in virology and the treatment of infectious diseases.

In addition to the identification and classification of a viral isolate, the genotype may be used to detect mutations in the genome that may have consequences with regards to viral infectivity, pathogenesis or response to different antiviral agents. The HCV genotype is an important predictor of response to interferon (IFN)- α based antiviral therapy [5, 6] and genotypes 1 and 4 are more resistant than genotypes 2 and 3 to the current standard of care. Patients with HCV genotype 6 gain a better response to combined-treatment with IFN- α and ribavirin than those with HCV genotype 1 [6]. No virological similarity between genotype 6 and 2 or 3 is reported and genotype 6 is not similar to genotype 2 or 3 by phylogenetic analysis [7].

The patterns of HCV genotypes can be identified throughout the genome by several methods [8]. However, it has been reported that mistyping between genotype 1 and genotype 6 could occur with the 5'UTR-Restriction Fragment Length Polymorphism (RFLP) [9] and some genotype 6 variants found in SEA have 5'UTR sequences identical to those of subtype 1a or 1b [10, 11]. Therefore, we analyzed HCV genotypes on sequencing of part of the core region of the genome followed by phylogenetic analysis in this study.

A higher prevalence of HCV infection ranging from 1.5% to 9% has been reported in SEA and the Indian subcontinent, with the highest rates of HCV (2-14%) present in northern and central Africa, the eastern Mediterranean, and Ukraine [12]. One study reported that among the HCC cases, 35% were positive for anti-HCV detected by using Anti-C100-3 EIA test kits [13]. The survey on HCV in Myanmar showed a high prevalence of hepatitis C infection in patients with thalassemia and in those with

liver diseases [14]. HCV infection is highly prevalent in Myanmar both in the apparently healthy population as well as in patients with end-stage liver diseases [15].

The previous study of HCV distribution in Yangon city was reported that three genotype 6 subtypes exist in Myanmar and according to the newly proposed nomenclature of hepatitis C, two subtypes (M6-1 and M6-2) belong to subtypes 6m and 6n respectively but one subtype has been unassigned yet [16]. Further to the reported study, we carried out the present study to investigate into the diverse distribution of HCV genotype 6. We assume that in border areas more diversity could exist as a result of human interlacing due to border trade and cross country migration. Thus, we aimed to analyze the HCV isolates in Tamu Township and to determine the genetic diversity of HCV isolates between Myanmar and neighboring country.

MATERIALS AND METHODS

Samples

A descriptive cross-sectional survey was conducted in Tamu Township, northwestern border area of Myanmar. A total of 501 peripheral blood samples were collected under informed consent. The sera were tested for anti-HCV antibodies by the Ortho HCV Ab-PA (Particle-Agglutination) Test II kit (Ortho-Clinical Diagnostic K.K., Tokyo, Japan) and stored in -80 °C.

RNA extraction

HCV RNA was extracted from 125 μ l of positive serum by single-step method of acid guanidinium thiocyanate-phenol-chloroform extraction [17] using Isogen-LS reagent (Nippon Gene Co., Toyama, Japan) according to the manufacture's instructions, and precipitated with isopropanolol and washed with 70% ethanol. The resulting pellet was resuspended in 20 μ l of RNase free water.

RT-PCR and nested PCR

Reverse transcriptase polymerase chain reaction and nested PCR were performed according to the method of Ohno T *et al.* [18] with minor modification. Briefly, extracted RNA was first amplified with outer primers Sc2 and Ac2 at a portion of core region in a 20 µl reaction volume. The second round PCR was carried out using 1 U AmpliGold *Taq*DNA polymerase (Roche Applied Science) and 1 µl each of inner primers S7 and A5. The final PCR products were resolved by 3% agarose gels and stained with ethidium bromide and evaluated under UV light. The genotype 6 new subtype was further analyzed by reverse transcription and nested PCR designated to an amplified portion of NS5B region of the viral genome by using outer (1S, 6AS) primers [16] and inner (1203, 1204) primers [19]. To avoid the risks of false positive results, the PCR assays were done with strict precautions against cross contamination.

Directs sequencing of PCR products

The second round PCR amplicons were purified with MonoFas DNA Kit I and proceeded to sequencing reaction with Big Dye Terminator 1.1 cycle sequencing reaction kit. Sequencing was performed using an ABI PRISM 310Genetic Analyzer (Applied Biosystems, Foster city, CA).

Sequence alignment and phylogenetic analysis

Multiple alignments of our sequences with the reference sequences on core/NS5B region of different genotypes retrieved from DDBJ/GenBank database were carried out. Alignment of the sequences and phylogenetic tree were constructed by using the neighbor-joining method based on genetic distances calculated using the Kimura two parameter substitution model, as implemented in MEGA version 3.1 [20]. To access the reliability of the phylogenies, bootstrap re-sampling and re-estimation were performed in 1,000 replications for neighbor-joining trees. The sequences

obtained in this study have been submitted to GenBank/DDBJ and have been assigned accession numbers from AB269327 to AB269352 (Core sequences), AB269353 (NS5B sequence).

RESULTS

Anti-HCV screening of all 501 subjects revealed 64 (12.8%) positive cases in Tamu Township. The mean age was 30.7 ± 15.4 years and 44.7% of the subjects were male. The prevalence of HCV infection by age, gender, and racial group is shown in Table 1. The prevalence rate by the 40-59 years age group was the highest (21.3%) and the lowest (2.9%) for the <20 years age group in this study. The youngest anti-HCV positive subject was 15 years old while the oldest was 67 years. Anti-HCV prevalence rate in male subjects was higher than female subjects. The prevalence rate in Chin race was highest (14.4%) among racial groups although Bamar population is the highest in the study. The higher rate (14.3%) in Narga race may be due to small number of subjects.

Table 1. Anti-HCV positivity with age, gender and racial group

Category	Number tested	Anti-HCV positive	Prevalence (%)
<i>Age group (yr)</i>			
<20	138	4	2.9
20-39	223	33	14.8
40-59	122	26	21.3
≥60	18	1	5.6
<i>Gender</i>			
Male	224	37	16.5
Female	277	27	9.7
<i>Racial group</i>			
Bamar	303	40	13.2
Shan	118	17	14.4
Chin	64	6	9.4
Narga	7	1	14.3
Others	9	0	0
Total	501	64	12.8

Among 64 samples, 59 samples (92.1%) could be amplified on the partial core genome and analyzed as three major genotypes and six minor subtypes named as 1a, 1b, 3b, 6m, 6n and new subtype of genotype 6. Genotype 6 (57.6%) accounted for the

majority of the samples, followed by genotype 3 (25.4%), and genotype 1 (17%). Neither genotype 2, 4 nor 5 was found. Subtype 6n was the most predominant subtype (52.5%), second most is subtype 3b (25.4%), followed by 1b (15.3%), 6m (3.4%), 1a (1.7%), and new 6 subtype (1.7%).

Table 2 shows the distribution of HCV genotypes stratified by age, gender and race. Most HCV genotypes were distributed in 20-39 years and 40-59 years age groups. Subtype 6n and 3b were predominant in 20-39 years age group and 40-59 years age group respectively. Subtypes 6n, 3b, and 1b were prevalent in male gender. All six HCV subtypes were distributed in Bamar, three subtypes in Chin and Gorahkar and only one in Narga. The most predominant genotype was subtype 6n and second most predominant was subtype 3b. The new genotype 6 subtype was found only in the Bamar race.

Table 2. HCV genotype distribution with age, gender and racial group

HCV geno- types (No)	Age group (yrs)				Gender		Race			
	<20	20 39	40 59	≥60	M	F	Ba- mar	Chin	Gora- khar	Nar- ga
1a(1)		1			1		1			
1b(9)	1	4	4		6	3	3	5	1	
3b(15)	1	4	9	1	9	6	6	6	3	
6m(2)	1	1			1	1	2			
6n(31)		19	12		18	13	23	5	2	1
6 [§] (1)			1	0	1		1			
Total (59)	3	29	26	1	35	24	36	16	6	1

[§] Referred to as new genotype 6 subtype
M=Male, F=Female

Fig. 1 shows that phylogenetic tree constructed on partial core sequences (nucleotides 380-659) along with sequences of different genotypes retrieved from GenBank /DDBJ database. HCV genotypes are indicated on the roots of cluster or individual branch. The bar at the base of the figure shows the scale for nucleotide substitution per site. The six HCV genotypes were indicated with numbers 1-6 and subtypes were designated 1a-6n.

DISCUSSION

In this study, we could characterize the three major HCV genotypes and six subtypes in Tamu Township with predominance of genotype 6. This finding was different with the genotype predominance of other reports [11, 16, 21]. That might be due to either the regional difference or transmission pattern of HCV infection in Myanmar. It may be probable that (i) the frequency of infection with genotype 6 group subtypes differs between geographical regions within Myanmar or (ii) there are differences in its distribution in different risk groups for infection or age of subjects or (iii) different genotyping methods or regions of genome were chosen to analyze. One sample of HCV isolate in this study could be provisionally assigned as a new subtype of genotype 6 by analysis on core and NS5B region, according to the consensus proposals for a unified system of nomenclature of HCV genotypes by Simmond P *et al.*

In fact, the routes of transmission of HCV infection or the factors involved were not identified in the present study. There was only a little evidence of history of tattooing or history of hepatitis in family. We could propose that HCV may be transmitted by the probable routes such as (a) blood transfusion in remote areas where HCV testing is not easily available (b) tattooing or ear piercing as religious practice, and (c) intravenous drug users with sharing needles. We are conducting further investigations to clarify the transmission route of this infection.

Comparing the genotypic pattern of HCV infection with neighboring country, HCV genotype distribution in Tamu Township was different from those in India. It has been reported that genotype 3 is the most prevalent genotype in India [22]. Some genotypes/subtypes may predominate within the regions and different HCV genotypes have unique patterns of geographic distribution [23]. The subtypes 1a, 1b, 2a, 2b and 3a are distributed globally and rarer,

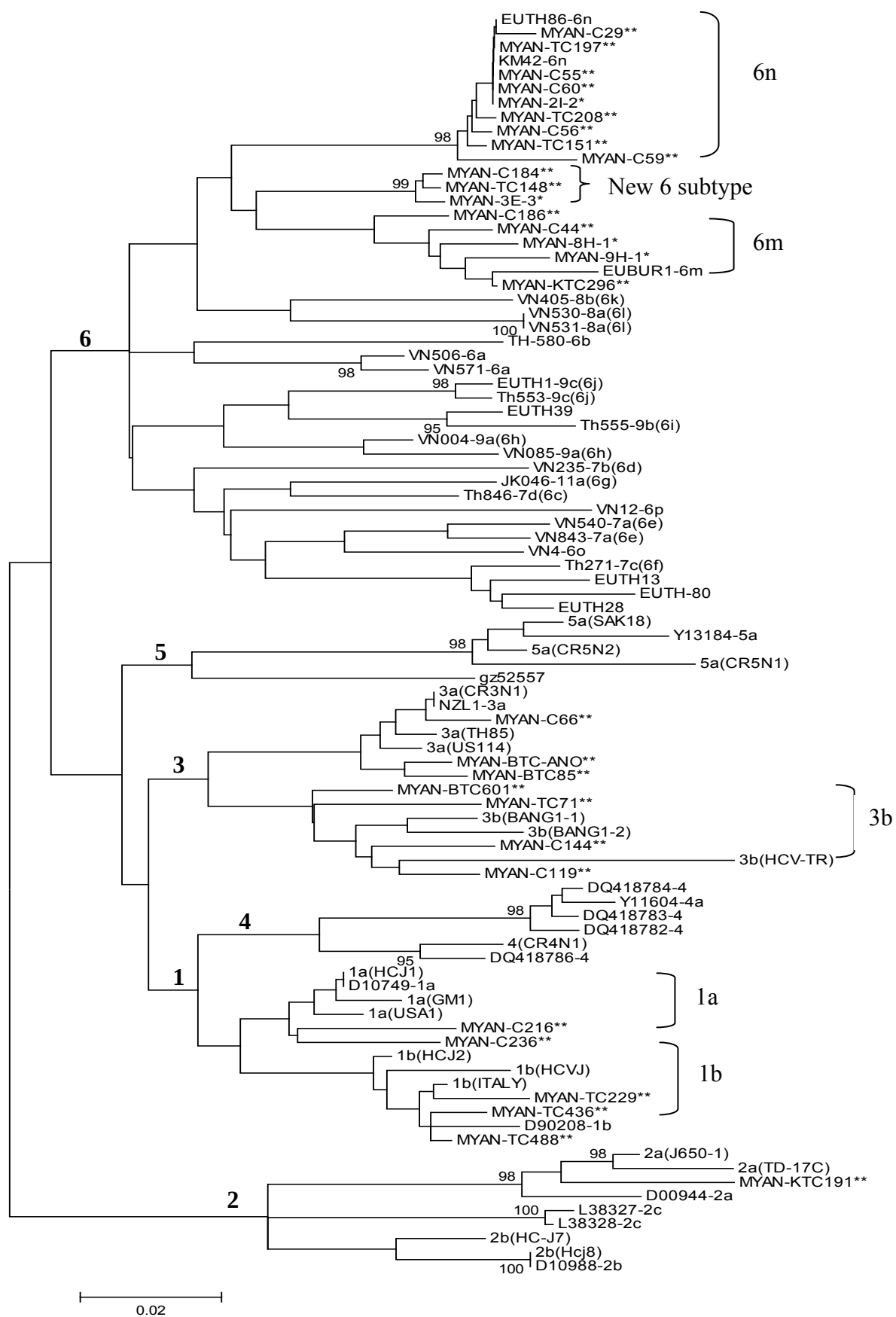


Fig.1. Phylogenetic tree constructed on partial core sequences (nucleotides 380-659) along with sequences of different genotypes

genotypes have also been localized in certain geographical regions. For example, subtype 3k has only been found in Indonesia [10], genotype 4 in the Middle East and Egypt [24], genotype 5 in South Africa [25], and genotype 6 and its variants in South-east Asia (SEA) [11, 26]. Racial heterogeneity exists firmly in Myanmar with eight major groups (Bamar, Shan, Kayin, Kachin, Mon, Yakhine, Chin and Kayar), 135 minor indigenous groups and those races of neighboring countries, predominantly China, India and Bangladesh. The Bamar indigenous people settled predominant in any region of Myanmar. Both indigenous group and non-indigenous group resided in border area. Bamar and Chin were two major indigenous groups in this study (Table 1). We have shown that HCV genotypes were more prevalent in the middle aged group and in Bamar and Chin indigenous group especially for subtype 6n and 3b. Although we could not postulate a definite relationship between HCV genotypes and age or racial group factors, we could provide a pattern of genotype distribution among different racial groups. Regarding the prevalence of HCV in this study, it was higher than those of the previous studies on the apparently healthy subjects [15] and the blood donors in Myanmar [16]. Although HCV prevalence survey could not be considered to be representative of the population at large, it could be concluded that significant higher rates of HCV infection exists among apparently healthy populations residing in border area. It is probable that the frequency of HCV infection will differ in age of subjects, different risk groups or transmission patterns of the infection but no risk factors could be explored in this study. Therefore, further large-scale in-depth studies will be needed to clarify the situation that will lead to the development of more effective prevention and control measures against HCV infection in this particular area.

Conclusion

This study highlighted that HCV infection was highly prevalent and various HCV

genotypes/subtypes with great genetic diversity of HCV genotype 6 were found in Tamu Township. In particular, the new genotype 6 subtype found in this study could be used to describe the epidemiology of HCV transmission in Myanmar and to enhance the understanding of the HCV sequence variation in SEA countries.

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**Preparation and standardization of an antimalarial
phytopharmaceutical product from *Swertia purpurescens* (Pan Kha)**

*Khin Phyu Phyu, **Win Myint, ***Aye Than, *Thein Tun, ***Ye Htut,
****Myint Sein, *Tin TinThein, *Lei Lei Win & *Saw Ohnmar Khin

*Department of Medical Research (Upper Myanmar)

**Department of Traditional Medicine

***Department of Medical Research (Lower Myanmar)

****Myanmar Pharmaceutical Factory

A standardized phytomedicine is locally produced from Pan Kha, *Swertia purpurescens*. Its antimalarial activity and the active principle were approved in 2004. The plant material was extracted with three different concentrations of ethanol; 50%, 70% and 96%. The 96% ethanol extract showed the presence of large amount of flavonoid (xanthone). This herbal drug was physicochemically and phytochemically standardized by using chromatographic and phytochemical methods for its herbal pharmacopoeial quality criteria. This phytomedicine was found to contain 7.82 mg % active principle, bellidifolin by HPLC analysis. The median lethal dose (LD₅₀) was determined as 37 (27.40-49.95) g/kg. Sub-acute toxicity test showed that there was no decrease in body weight of albino rats. No significant changes in weight of the internal organs, the biochemical and haematological profiles of the test groups were observed when compared with the control group. These findings call for a clinical trial of this medicine to be tested on human being.

INTRODUCTION

Myanmar traditional plant, Pan Kha (*Swertia purpurescens*, Gentianaceae) has been used by local people as an antimalarial remedy for many years in Loikaw, Kayah State. Many plants in traditional medicine are usually recognized as major resources of drug development by scientists and researchers. Several disciplinary research works on plants had been done and are still being preceded for their applicability as pharmacological tools. Then, the promising pharmacologically active molecules which can be synthesized in large scale will be used in solving many health problems. An entirely natural pharmaceutical product which contains an active compound together with other supporting compounds (assuming as naturally related compounds for combination therapy), will be very interesting to develop in a finished oral dosage form and

to be assessed by clinical trial. The plant, *Swertia purpurescens* had recently focused on *in vitro* and *in vivo* tests for antimalarial activity, acute toxicity test and chemical investigation for identifying an active principle [1]. At this stage, a research to develop a pharmaceutical herbal extract is called for, which can be applied in malarial treatment. A standardized total alkaloid extract namely totaquine produced from cinchona bark has been one of its kind for the treatment of malaria [2]. Development of a standardized xanthone extract from *Swertia* plant grown in Myanmar will be innovative approach of proving flavonoid as antimalarial remedy apart from an alkaloid or sesquiterpene.

MATERIALS AND METHODS

Swertia purpurescens (Pan Kha) was collected from Loikaw in November. The

whole plant was used for the preparation and standardization of extract. All solvents except locally produced ethanol are of analytical grade reagents from E.Merck, Darmstadt, Germany. Excipients (lactose monohydrate, dicalcium phosphate dihydrate and magnesium stearate) are the pharmaceutical grade products of Germany, China and Thailand.

Preparation of phytopharmaceutical product from plant extract

Before extraction, plant sample was air-dried and cut into small pieces. Exactly 100 g of sample was packed and subjected to solvent extraction by soxhlet assembly. This sample was extracted with three different ratio of ethanol, 50%, 70% and 96% respectively. The extraction time for each extract was six hours. Then the extracts were evaporated to concentrate by means of rotatory evaporator. It was followed by drying in air until solid residues were obtained. These residues were desiccated, weighed and their respective yields of extracts were calculated. The resultant three solid extracts were stored in desiccators. Then these extracts were checked with thin layer chromatography (TLC) for the presence of active principle, flavonoid (xanthone).

The 96% ethanol extract showed the presence of highest amount of flavonoid (xanthone). For large scale production, plant sample was percolated with 96% ethanol for one month. That extract was partitioned with petroleum ether to remove fatty matters and checked with TLC for active ingredient. After removing fatty matters, the extract was dried in air and then it was mixed with pharmaceutical excipients by using a few amount of ethanol and finally dried in air. Trial and error of 12 series of experiments were carried out by mixing varying ratios of three excipients until a thoroughly dried stage of solid powder was obtained. The specific amount of powder was used in capsule filling. Phytopharmaceutical standardization of the prepared powder was also done under controlled

condition. In order to have quality assurance of the samples of every batch, TLC scanner (CAMAG TLC scanner III), was used to confirm the presence of active ingredient, bellidifolin.

Standardization of the prepared phyto-medicine

The finished product was standardized by general analytical and pharmacopoeial methods (Quality control method for medicinal plants material, W.H.O (1998) [3], Indian Pharmacopoeia (I.P) (2001) [4], and British Pharmacopoeia (B.P) (1996) [5]. The quantity of active ingredient was measured by HPLC.

Validation of high-performance thin-layer chromatography method for determination of bellidifolin in herbal extract and pharmaceutical dosage form

A new, simple, sensitive, precise and robust high performance thin-layer chromatographic (HPTLC) method for analysis of bellidifolin was developed and validated for the determination of bellidifolin in herbal extracts and in pharmaceutical dosage form. Analysis of bellidifolin was performed on TLC aluminum plates pre-coated with silica gel 60F-254 as the stationary phase. Linear ascending development was carried out in twin trough glass chamber saturated with mobile phase consisting of n-hexane-ethyl-acetate (3:2v/v) at room temperature ($25 \pm 2^\circ\text{C}$). CAMAG TLC scanner III was used for spectrodensitometric scanning and analysis in absorbance mode at 237nm.

Acute and sub-acute toxicity tests

Acute toxicity test

Eight groups of 10 adult mice of both sexes weighing 25 ± 5 g were kept in cages of five each. The mice were treated with seven different doses i.e. 4, 8, 16, 20, 24, 28 and 32 g/kg of prepared antimalarial phytopharmaceutical product dissolved in water by using feeding nozzle. They were observed daily for one week. The lethality (LD_{50}) was calculated using the method of Lichfield & Wilcoxon [6].

Sub-acute toxicity test

For sub-acute toxicity test, four groups of six albino rats (Wistar strain), each weighing 140 ± 10 gm of both sexes were kept in separate cages. The rats were treated with 1 g/kg, 2 g/kg and 4 g/kg antimalarial phytopharmaceutical product using feeding nozzle for two weeks [7, 8, 9]. They were closely observed for two weeks. After that they were held another 24 hours before sacrificed. Then, the animals were sacrificed; the blood and the internal organs were collected.

Laboratory investigations

After two weeks of drug administration, biochemical tests, liver function tests and haematological tests (complete picture) were done and histopathological examination were made on heart, liver, lung, kidney, spleen, stomach and small intestine by a competent pathologist.

Statistical analysis

Comparative data of control and experimental animal test groups investigated in sub-acute toxicity test was analyzed by using Student's *t* test.

RESULTS

Yield percentage (w/w) of alcoholic plant extracts and corresponding defatted sample

The yield percentages of 96%, 70% and 50% alcoholic plant extracts were 18.1%, 19.1% and 11.2% respectively. Among them, 96% ethanol extract showed large amount of active principle, bellidifolin (Fig.1). After partition of the 96% alcohol extract of the plant with petroleum ether in order to remove fatty matter and to obtain naturally dry extract powder, the extract of defatted sample was 11.0% and the fat content was 7.1 %. Then, it is required to add or mix with an appropriate pharmaceutical excipient. Various kinds of pharmaceutical excipient which are available were tested for the chemical properties to attain dry extract

powder. The ratio of extract and excipients are 1:1.



Fig. 1. The prepared capsule

Determination of active compound from prepared phytomedicine

Active ingredient (AI) bellidifolin from the prepared phytomedicine was determined to be 7.82 % by HPLC analysis. A capsule contained 400 mg of phytopharmaceutical powder in which 200 mg was active plant extract. 31.28 mg of the flavonoid, bellidifolin was found to contain in a capsule.

Table 1. Pharmacopoeial specifications and quality criteria of capsule

Test parameter	Results
Appearance	Dark brown powder in red hard gelatin capsule
Average weight	400 mg/ cap
Uniformity of weight of capsule	Comply with Indian and British Pharmacopoeias
Disintegration time (limit not more than 15 mins)	(6-9) mins

R_f value of bellidifolin and analyzed spots of upper and lower layers of Swertia purpureus extracts

The same *R_f* values of defatted (2) and active ingredient, AI, 0.6. Upper layer of fat sample 1 was found to be lack of bellidifolin while lower layer 2 showed the presence of bellidifolin (Fig. 2). The HPTLC analysis was found to give compact spots for bellidifolin in pharmaceutical form batch 1 to 5, *R_f* value of 0.6 ± 0.01 (Fig. 3).

In the acute toxicity test, (*LD*₅₀) was determined as 37 (27.40 - 49.95) g/kg. In sub-acute toxicity test, all tested rats were maintained the normal physical appearance

Table 2. Physico-chemical data of finished drug powder

Test parameter	Results
<i>Organoleptic characteristics</i>	
Appearance	powder
Colour	dark brown
Odour	characteristic
Taste	very bitter
Loss on drying at 105 °C	4.0%-4.30%
<i>Ash values</i>	
Total ash (%w/w)	5.30-6.25
Water soluble ash (%w/w)	0.5
Acid insoluble ash (%w/w)	0.35
<i>Extractable matters</i>	
Water soluble matters (hot –extract)	75-80 %
Ethanol (96%) soluble matters (cold maceration)	40-45 %
<i>Bitterness value</i>	20.50 unit/g
<i>Foaming index</i> (height 16 cm, diameter 6 mm)	250-333.3

TLC data of reference bellidifolin and *Swertia purpurescens* extracts

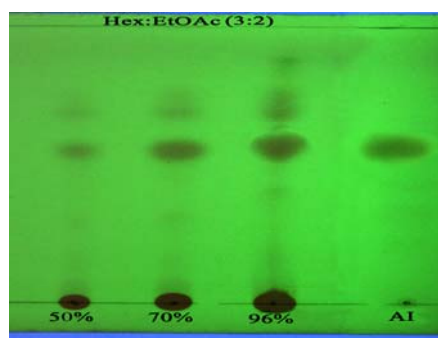


Fig. 2. Thin layer chromatogram of 50%, 70% and 96% *Swertia purpurescens* ethanol extracts and active ingredient (AI)

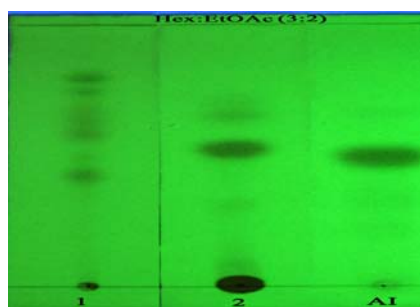


Fig. 3. Thin layer chromatogram of upper (1), lower layer (2) and active ingredient (AI)

and there were no gastrointestinal side-effects during the administration of drug observed. The body weight gains in all the

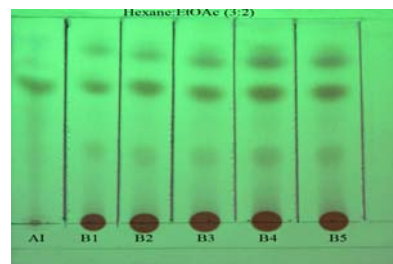


Fig.4. Active ingredient (AI) and batch 1 to 5 of finished drug, solvent system, Hexane: EtOAc (3:2 v/v)

three tested groups were similar to the control group. No significant changes in weight of the internal organs such as heart, liver, lung, kidney, spleen, stomach and intestine were observed.

Laboratory investigations

No significant differences in biochemical, liver function and haematological parameters were observed between test and control groups of laboratory animals. Mild congestions were marked in kidney and lung at the high dose of 4g/kg.

DISCUSSION

The study brings out a promising herbal product, which will hopefully be useful for the treatment of malaria. This phytomedicine has actually been developed basically on Myanmar traditional therapeutic uses and experimentally proven data. In the first phase of the project, *in vitro* as well as *in vivo* antimalarial activity and related active chemical constituent of the *Swertia purpurescens* had been already reported in 2005 [1]. This second phase has dealt with preparation of standardized herbal extract from this plant by applying phytopharmaceutical technology. This phytomedicine also fulfills the needs of scientific approach to traditional medicine by the virtue of its easy manufacturing process, ready to use, economically affordable and therapeutically reliable for treating malaria. It is expected that this phytochemical compound would be applicable as reference substance for further production of this phytomedicine. The results of acute and sub-acute toxicity

studies have also indicated its safety. Now a scientifically proven herbal extract medicine had been already prepared and determined its quality, safety, and experimental effectiveness. Therefore, the authors would like to commence and encourage to do a case-control clinical trial research for the practical applicability of this standardized herbal medicine and that only thing necessary and last phase is to assess its “efficacy on human”.

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The role of immunohistopathology and polymerase chain reaction (PCR) in the diagnosis of leprosy

*Khin Saw Aye, *Aye Aye Win, *Yin Min Htun, *Tin Zar Maw,
Kyaw Kyaw & *Khine San Yin

*Immunology Research Division
Department of Medical Research (Lower Myanmar)
**Department of Health
***University of Medicine (1)

The aim of the study was to determine the role of immunohistopathology and polymerase chain reaction (PCR) in the diagnosis of leprosy. A total of 112 skin biopsy specimens from leprosy patients attending the Central Special Skin Clinic, Yangon General Hospital have been examined by hematoxylin and eosin (H&E) staining, modified Fite-Faraco (F-F) technique for *M. leprae*, immunostaining with the antibody against the phenolic glycolipid-1 (PGL-1) and Bacille Calmette-Guerin (BCG) using Avidin Biotin Complex (ABC) method and polymerase chain reaction (PCR) using the primers amplifying the 130 base-pair fragment of the gene from the 16S ribosomal RNA of *Mycobacterium leprae*. Based on the clinical diagnosis and histological (Ridley-Jopling) classification, indeterminate (ID) and tuberculoid (TT) types were the most common in paucibacillary (PB) cases (64.9%) and borderline lepromatous (BL) and lepromatous (LL) types were most common in multibacillary (MB) cases (58.7%). So, H&E stain still plays an important role in the histo-pathological examination and is still the basic method for diagnosis and categorization of leprosy. Fite-Faraco stain was negative in all PB cases and half of the MB cases were positive. The finding was consistent with the clinical diagnosis of leprosy. According to the histological classification, detection of *M. leprae* by Fite stain was 100% negative in ID and TT types. Fite stain positivity was lowest in ID type (0%) and highest in LL type (94%). So F-F stain positivity correlated with the histological types of leprosy. F-F, BCG, and PGL-1 staining compared to PCR provided virtually 100% specificity, but only 40%, 50% and 46% sensitivity in detecting the organisms in clinical samples respectively. Application of histopathology, histochemistry, immunohistopathology and molecular techniques (PCR) for identification of *M. leprae* was proved to be useful to diagnosis of early leprosy, which is important to maintain the achievements of Leprosy Elimination Program in Myanmar.

INTRODUCTION

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, an acid-fast, rod-shaped bacillus. The disease mainly affects the skin, the peripheral nerves, mucosa of the upper respiratory tract and also the eyes, apart from some other structures. Currently, global situation of leprosy prevails mainly in a limited number of countries in Africa, Asia and

South America. The strategy to eliminate leprosy as a public health problem (defined as a registered prevalence rate of less than 1 case per 10,000 population) has helped countries to further reduce the burden of disease. WHO will continue its support to the remaining highly endemic countries, to ensure that the goal of elimination is achieved. In addition, it will provide essential support to countries that have achieved elimination, to reduce the disease

burden further and to ensure that the leprosy control services are sustained, by providing technical support, monitoring the leprosy situation, providing MDT drugs free of charge, improving drug supply logistics and promoting advocacy to maintain political commitment at all levels [1].

In Myanmar, leprosy stands at the 8th position of our national health priority diseases (2001) [2]. Currently, leprosy elimination has been achieved at the national level at the end of January 2003. The prevalence rate (PR) at the end of June 2003 was lower than 1 per 10,000 population in all states and divisions [3].

After complete elimination of leprosy in the year 2003, there should be no full-blown cases of leprosy developing. Therefore, early diagnosis and prompt treatment is very important to prevent nerve damage and development of deformities. The diagnosis of early leprosy, especially single-lesion leprosy, based upon clinical and skin smear examination is often difficult under field conditions as well as in leprosy clinics. Therefore, in clinically doubtful cases, confirmation of diagnosis is essential by performing a skin biopsy to detect early histopathological lesions, supplemented by special histochemical, immunohistochemical and molecular techniques.

In Myanmar, a large number of research for dissertations and theses have been done on leprosy problem in many fields of study such as Microbiology, Pharmacology, Paediatrics, Internal Medicine, Public Health, Preventive and Tropical medicine, Ophthalmology and Dermatology. However, early tissue changes in leprosy, immunohistopathological techniques and molecular studies have not been conducted.

In this study, routine conventional histopathology using H & E stain, special stain using F-F, IHC using BCG and PGL-1 antibodies and PCR methods were performed on skin biopsies with the hope of getting an early diagnosis of leprosy.

MATERIALS AND METHODS

This study is a descriptive, cross-sectional, hospital and laboratory-based study and was carried out from year 2003 December to 2006 June at Immunology Research Division, Department of Medical Research (LM) and Central Special Skin Clinic, Yangon General Hospital, Yangon. One hundred and twenty cases of leprosy patients attending the CSSC, YGH were included. Diagnosis and classification of leprosy was done according to WHO criteria [4].

Ethical clearance was taken from Ethical Review Committee, DMR (LM). The research procedures were explained thoroughly to the patients before taking the informed consent. A thorough history taking and physical examination were done and the findings were recorded in the proforma. Slit skin smear from skin lesions and a skin biopsy were taken. Biopsy specimens were cut in half; one half was used for paraffin embedding, and the other half was preserved in 70% ethanol used for PCR.

The following methods were applied to serial sections: hematoxylin and eosin (H&E), Fite's acid-fast method and immunohistochemical staining by using ABC technique [5]. The primary antibody against PGL-1 monoclonal was provided by Dr. M. Makino, NIID, Japan using dilution of 1:1000 [6] and antibody to BCG polyclonal derived from *M. tuberculosis* (DAKO), used at a dilution of 1:2000. They were applied for 30 minutes at room temperature. Counter stain was done with Mayer's hematoxylin. Preparation of *M. leprae* DNA from biopsy specimens was done using QIAGEN (Germany) kit. Amplification of *M. leprae* DNA by PCR using primers corresponding to portions of the sequence of 16SrRNA of *M. leprae* and generate a 130-bp fragment. The sequences of primers were:

ML16S: 5'AAAAAATCTTTTTTAGAGAT 3'

ML16SA: 5'TTCAAGGCGCATGTCTTG 3'

The 50 µl reaction mixture contained 10 µl of template solution, 0.2 µl of *Ex Taq* DNA polymerase (Takara Shuzo Co., Shiga, Japan), 1 µM of each primer, 5µl of 10x DNA PCR buffer, 8 µl of dNTP solution and 25.8 µl of water. The reaction was performed with a Mastercycler personal eppendorf AG, Hamburg, Germany. The reaction mixture was heated to 94°C for 1 min, rounds of amplification consisted of a 30-second denaturation step at 94°C, a 2-min annealing step at 44°C and a 3-min elongation step at 72°C for 45 cycles. Ten microliters of the reaction mixture were electrophoresed on 2% agarose gels. After electrophoresis, the gel was stained with ethidium bromide, and the 130 bp DNA band was examined under UV illumination.

Reporting of results

1. Histopathology results

Histopathology results were reported as indeterminate (ID), tuberculoid (TT), borderline tuberculoid (BT), mid borderline (BB), borderline lepromatous (BL) and lepromatous (LL) types according to Ridley-Jopling classification.

2. Histochemical results

F-F stain results were reported as positive and negative. *Mycobacterium leprae* are pink rod-shaped organisms measuring approximately 3 to 8 µ in length. The acid-fast organisms were seen against a blue background lying singly, as cigar shaped bundles, or as globi in positive cases.

3. Immunohistochemical results

Immunoexpression of BCG antigen and PGL-1 antigen were reported as positive and negative, the degenerated bacilli can be seen as brown color inside the foamy macrophages in positive results.

4. PCR results

Identification of *M. leprae* DNA by PCR method was reported as positive and negative. The samples were analyzed by electrophoresis on 2% (wt/vol) agarose gels

for the presence of DNA bands. The positive cases for *M. leprae* DNA were seen as DNA bands at the 130bp level of molecular weight markers and at the same site as positive control. There were no DNA bands at the 130bp of molecular weight markers for the negative results (Fig.1).

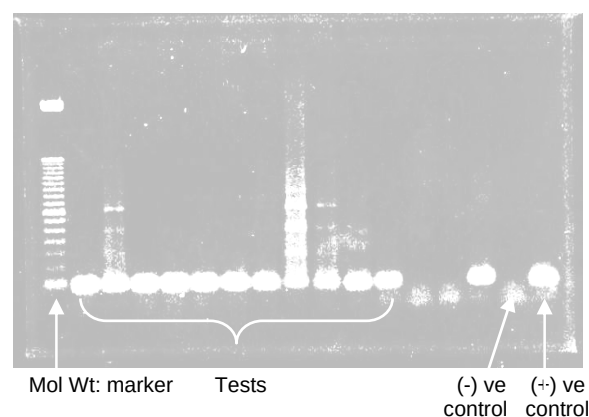


Fig. 1. Gel electrophoresis of PCR product for amplification of 130bp DNA of *M. leprae* from biopsy tissue. Lane 1, Molecular wt: marker (100bp), lane 2 to 12 and 15 positive cases, lane 13 and 14 negative cases, lane 17 positive control (Thai 53 strain) and lane 16 negative control.

RESULTS

A total of 112 skin biopsy specimens from leprosy patients attending the CSSC, YGH were studied during 18 months period from June 2004 to December 2005.

Age

Minimum age was 5 years and maximal age was 78. Mean was 47 with standard deviation of 15.75. Mode was 50.

Clinical types of leprosy by WHO classification

The patients were clinically classified by WHO criteria as PB and MB, which constituted 33 % and 67% respectively.

WHO and Ridley-Jopling classifications

Based on both clinical (WHO) and histological (Ridley - Jopling) classification of leprosy, ID type was most common in PB

cases and LL type was most common in MB cases (Table 1).

Table 1. WHO and Ridley-Jopling classifications

WHO classification	Ridley-Jopling classification									Total
	LL	BL	BB	BT	TT	ID	Normal	NSD	Healed	
PB	0	0	1	6	6	18	6	0	0	37
MB	17	13	5	9	11	13	2	2	3	75
Total	17	13	6	15	17	31	8	2	3	112

Positivity of lepra bacilli by F-F stain in clinical types of leprosy

All PB cases were F-F stain negative (100%) and 57.3% of MB cases were F-F positive. AFB was negative in slit skin smears of PB cases.

Histopathological types and detection of *M. leprae* by F-F, BCG, PGL-1 and PCR

Fig. 2 shows histopathological types and positive detection of *M. leprae* by F-F, BCG, PGL-1 and PCR. Positive results of F-F were lowest in ID type and positive results of PCR were highest in LL type.

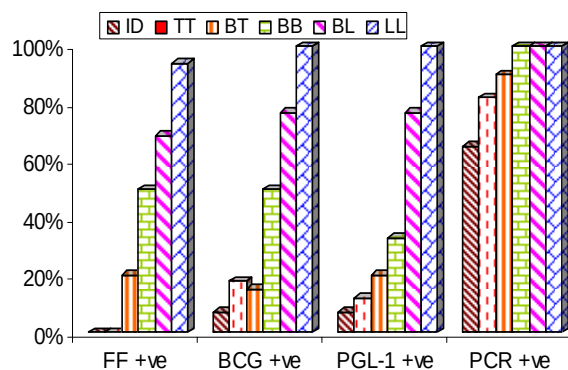


Fig. 2. Histopathological types and positive detection of *M. leprae* by FF, BCG, PGL-1 and PCR

Comparison of agreement rates between PCR and F-F, BCG, PGL-1

For comparison of agreement rates between PCR and F-F, BCG, PGL-1, agreement rate of F-F was 53.6%, PGL-1 was 60.71% and BCG was 65.17%. BCG was the best test among 3 tests compared with PCR by agreement rates.

Identification of *M. leprae* in skin biopsy by F-F and PCR

All 30 AFB positive cases by Fite's method were PCR positive. This study also showed that PCR was always successful in detecting *M. leprae* in clinical biopsy samples which were microscopically confirmed to have the organisms. The overall agreement between PCR and microscopic examination in detecting *M. leprae* using F-F was 53.6%. There was highly significant difference between PCR and microscopic examination for the detection of *M. leprae* in biopsy specimens ($P = 0.0001$, <0.01 , t-test).

Identification of *M. leprae* in skin biopsy by PGL-1 and PCR

All of 37 PGL-1 antigen positive samples were also PCR positive. Among 75 PGL-1 antigen negative samples, 44 (59%) were PCR positive and 31 (41.3%) were PCR negative. There was significant difference between PCR and PGL-1 antigen ($P = 0.001$, <0.01 , t-test) for the detection of *M. leprae*.

Identification of *M. leprae* in skin biopsy by BCG and PCR

All of 40 BCG antigens positive samples were PCR positive. Among 72 BCG antigens negative samples, 39 (54.2%) were PCR positive, 33 (45.83%) were PCR negative. There was significant difference between PCR and BCG antigens ($P = 0.0001$, <0.001 , t-test) for the detection of *M. leprae*.

Table 2. Sensitivity, specificity and predictive values (PV) of F-F, BCG and PGL-1 stain compared to PCR

Tests compared to PCR	PCR				
	Sensitivity	Specificity	Positive PV	Negative PV	Kappa
	95% CI	95% CI	95% CI	95% CI	95% CI
F-F	0.398	1	1	0.412	0.281
	(0.299-0.505)	(0.901-1.000)	(0.896-1.000)	(0.13-0.518)	(0.13-0.433)
	0.506	1	1	0.458	0.345
BCG	(0.398-0.614)	(0.896-1.000)	(0.912-1.000)	(0.348-0.573)	(0.192-0.498)
	0.457	1	1	0.423	0.292
	(0.533-0.565)	(0.89-1.00)	(0.908-1.000)	(0.309-0.526)	(0.14-0.433)

Sensitivity, specificity and predictive values (PV) of F-F, BCG and PGL-1 stain compared to PCR

Table 2 shows sensitivity, specificity and predictive values (PV) of F-F, BCG and PGL-1 stain compared to PCR.

DISCUSSION

Mycobacterium leprae, the etiologic agent of leprosy remains one of the few pathogens that can not be cultivated *in vitro*. The diagnosis of leprosy is still based upon principles used a century ago, a clinical examination of the patient's lesions, demonstration of acid-fast bacilli (AFB) in slit-skin smears, and histopathology. Leprosy is most easily diagnosed when *Mycobacterium leprae* are demonstrable in diseased tissues, but this is often difficult in the indeterminate and tuberculoid types of leprosy where *M. leprae* is rarely detected. Histopathological diagnostic procedures allow histological categorization of different types of leprosy but it is relatively insensitive as a definitive identification of the infecting organism when *M. leprae* is not positive with routine H&E staining. Several attempts have been made in recent years to improve the sensitivity and specificity for the detection of *M. leprae* with Immunology, Biochemistry and nuclei acid probes [7]. For example, Clark-Curtiss and Docherty [8] have described a DNA probe that can be used in a dot blot hybridization assay to detect as little as 1pg of purified DNA, the amount in approximately 300 bacilli.

Recently, many scientists have studied the use of polymerase chain reactions (PCR) to detect mycobacteria. This method has been used to detect extremely low numbers of *M. leprae* in fresh unfixed human skin biopsy specimens, providing a powerful direct and unequivocal test for *M. leprae* infection [9]. In the literatures, there have been various target sequences for PCR and DNA probes specific for *M. leprae*, such as genes encoding the 36-kDa antigen [9],

18-kDa antigen [10] or repetitive sequences of *M. leprae* [11] and 16S ribosomal RNA of *M. leprae* [12]. Most of the reports showed that PCR with DNA probes seemed very sensitive, so that even 1 to 100 organisms were detectable by the method. In addition, PCR provided virtually 100% specificity in detecting the organism in clinical samples [13].

At the same time, the demonstrations of phenolic glycolipid-1 (PGL-1) as a *M. leprae*-specific antigen and of Bacille Calmette-Guerin (BCG) as a common antigen in all species of *Mycobacterium* in skin biopsies were done by immunostaining using Avidin Biotin Complex (ABC) technique. This study has attempted to compare the diagnostic accuracy between immunostaining method and PCR-based molecular method in *M. leprae* infection of various clinical settings and then to evaluate the role of these tests in the early diagnosis of leprosy.

All of the 112 patients included in the study had a similar clinical picture, having one or more hypopigmented macules mostly on the extremities with minimal or no impairment of sensation, or having skin lesions with impairment of sensation. Based on clinical classification (WHO), 75 cases (67%) were MB and 37 cases (33%) were PB. Regarding the histological (Ridley-Jopling) classification, ID type was the most common in PB (18 cases) and LL type was most common in MB (17 cases). So H&E staining still plays very important role in the histopathological examination and is the basic method which should always be done in the routine work on leprosy diagnosis. Liu, *et al.* [14] reported that in the study of histology in indeterminate leprosy, involvement of the dermal nerves, with perineural infiltration being the most common finding. Porichha *et al.* [15] has stated that the presence of AFB and nerve involvement were taken as definite features for a diagnosis of leprosy, and infiltration of the dermal appendages, neurovascular bundles and dermis by epithelioid cells and

lymphocytes were regarded as suggestive signs of leprosy.

By using Fite-Faraco stain, *M. leprae* were found in the subepithelial connective tissue, in the macrophage of granuloma in the dermis, and in dermal nerves. Further, dermal nerves also showed destruction of the entire perineurium with much of the endoneurium being preserved. In a case of early leprosy with a single skin lesion, proliferation of perineurial cells with AFB in one of them and no significant endoneurial changes has been reported [16]. This study also confirmed Ridley's contention that in all biopsies of patients with early leprosy, AFB could be detected if fairly large numbers of serial sections stained properly for *M. leprae* were carefully examined under oil immersion lens and searched for in the appropriate sites. Fig.2 shows Fite stain positivity was lowest in ID type and highest in LL type. So F-F stain positivity was correlated with histological types of leprosy. Fite stain for *M. leprae* has confirmed the clinical diagnosis of leprosy.

Lejbkiewicz *et al.* [17] reported that leprosy is rare and non-endemic in Israel, cases of leprosy were invariably imported by immigrants or foreign workers and pathologists are not always alert to the possibility of leprosy or recognize potential symptoms. Therefore it is recommended that confirmation of leprosy should be done in all cases by histopathology of suspected lesions supplemented with staining for acid-fast bacilli by modified Fite-Faraco staining. PGL-1 is a species-specific antigen of *M. leprae* [18].

In contrast to it, BCG is revealed as a common antigen in all species of the *Mycobacterium* [19]. The specificity of the immunohistochemical staining using the antiserum against PGL-1 antigen on formalin fixed and paraffin embedded leprosy skin biopsy specimens has been proved by Goto *et al.* [20]. The specificity of the immunohistochemical staining using the antibody against BCG is just similar to that of acid-fast staining. As for the

sensitivity, the result indicated that the sensitivity of the demonstration of the PGL-1 antigens was more than that of the demonstration of the BCG antigen [20]. It is probably due to the amount of BCG and the amount of PGL-1 accumulated in the skin lesion. Both PGL-1 antigen and BCG antigen were demonstrated in some nerve bundles, erector pili muscles, epithelioid cells and endothelial cells of blood vessels where AFB were absent. The conventional acid-fast staining such as Fite's method can demonstrate *M. leprae* with acid-fast staining features only. But the above procedures all fail to demonstrate the soluble antigen released from *M. leprae* [19].

However immunohistochemical staining can identify both the bacilli and released soluble antigens in the vacuolar pattern [19]. It is useful and very important for the demonstration of *M. leprae* in early lesion and regressive lesions particularly in paucibacillary cases. In this study BCG was positive in 2.7% and PGL-1 was positive in 8.1% among PB cases. Among MB cases, the BCG was positive in 50.7% and PGL-1 was positive in 45.3%. Fig. 2 shows higher positive rates of PGL-1 and BCG than F-F stain in all types of Ridley-Jopling histological classification ie., ID, TT, BT, BB, BL and LL by microscopic examination. The detection of bacillary antigens, together with the clear demonstration of nerve bundles enhanced the capacity to fulfill morphologic criteria for the diagnosis of early leprosy. These observations indicated that immunohistochemical methods represented useful tool for the early diagnosis of leprosy.

In this study, the positive rate of BCG was higher than that of PGL-1 in both PB and MB cases as well as in TT, BT and BB types. Deps *et al.* [21] reported that false positive reaction of the immunohistochemistry technique using anti-BCG polyclonal antibodies to identify *M. leprae* in wild nine-banded armadillos was identified. In our study, positive and negative controls were tested simultaneously

with patients' specimens. Results were always compatible with controls. However, basal cells of skin, fibroblast and endothelium cells were showed BCG positive. The positive rate with BCG was found to be higher than with PGL-1. This may be due to false positive reaction as reported by other study [21]. Therefore, the diagnosis of leprosy should be done with histopathological study supplemented by other IHC test like PGL-1 and AFB stain with Fite-Faraco. PCR was 100% positive in BB, BL and LL types of leprosy and 64.5%, 82.4% and 60% positive in ID, TT and BT types. These findings confirm the previous reports of others that PCR has high sensitivity and near perfect specificity to detect *M. leprae* [10, 22, 23, 24].

Agreement rates between PCR and F-F, BCG and PGL-1 stain were also 53.6%, 65.17% and 60.7% respectively. BCG stain showed higher sensitivity compared to the other two tests, F-F and PGL-1 stain. Table 2 shows sensitivity, specificity and positive and negative predictive values of F-F, BCG, and PGL-1 stain with PCR, 100% specificity in all tests and 40%, 50% and 46% sensitivity in FF, BCG and PGL-1 stain. Scollard, *et al.* [25], Qinxue *et al.* [26] and Haque *et al.* [27] have also reported the specificity of the PCR was 100% in their clinical referral material. Sensitivity ranged from 50% to 83%. The assay is highly informative when acid-fast bacilli are not detectable by light microscopy.

Job CK *et al.* [28] has reported that even in endemic countries the profile of leprosy was changing, and detection of leprosy lesions in their early stages has become increasingly important. Since the finding of *M. leprae* is crucial in the confirmatory diagnosis of early leprosy, it is suggested that PCR studies to detect *M. leprae* should be done wherever possible in conjunction with histopathologic examination. It is also recommended that the feasibility and the cost-effectiveness of both of these methods to find *M. leprae* be evaluated.

In this study, the PCR showed highest positive rate in all histological types. The *M. leprae* specific 130 bp pair fragment was amplified by using the primers specific for the nucleotide sequence from No. 81 to 210 which include the V1 region of *M. leprae* 16 SrRNA gene. Cox *et al.* [12] have found the sequence of this region consisting of 12 extra nucleotides (6As and 6Ts) which represents a unique *M. leprae* sequence compared to the other mycobacterial species sequences. It can be used to design PCR primers that provide a rapid, unequivocal and non-radioactive test for the presence of *M. leprae*. A primer based on this sequence was used in a PCR protocol for the detection of *M. leprae* and the PCR product showed appropriate size with cloned *M. leprae* DNA, with purified genomic DNA and with various *M. leprae*-infected tissues, but not with other mycobacterial species. The group also showed that there were significant differences between the *M. leprae* 16 SrRNA sequences and other mycobacterial sequences [12].

These differences could be exploited to detect and identify *M. leprae*-infected tissue. In this study, the 130-bp DNA product amplified by PCR using primers ML16S and ML16SA was obtained only from *M. leprae* and not from other 16 mycobacterial species. The finding supported that PCR procedure provides an advantage of not requiring hybridization using a DNA probe or any other step to confirm that the 130-bp DNA product is specific to *M. leprae*.

With the availability of numerous effective anti-leprosy drugs, leprosy is being rapidly controlled throughout the world. The profile of leprosy patients presenting in an out patient clinic is also radically changing, and more and more early lesions are encountered. Therefore, any additional tool for use in detection will be a great advantage in the field of early diagnosis. Detection of *M. leprae* using PCR methodology has recently emerged as a diagnostic tool. It is, at present, too elaborate and

expensive to be used in routine diagnostic work. Improving the technique of PCR study and making it cheaper is imperative.

Conclusion & recommendation

The PCR assay was also useful as a tool for detection and follow-up of possible leprosy cases. It could be used to monitor high-risk populations and thereby maintain the achievements of leprosy elimination programs in countries where the disease's prevalence has been significantly reduced. Early diagnosis and prompt treatment is very important for any infection including leprosy to prevent deformity and stigmatizations of patients. So PCR technique needs to be established at reference laboratory as National Health Laboratory and special stain (Fite-Faraco) and introduction of immunohistochemical stain facilities in the laboratory referred center as CSSC, YGH. It could be used to monitor high-risk populations and also to maintain the achievements of leprosy elimination programs in Myanmar, where the disease prevalence has been significantly reduced.

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Compliance of basic health staff on national anti-malarial treatment guideline in selected townships of upper Myanmar

**Hla Soe Tint, *Thein Tun, **Thar Tun Kyaw,
*Moe Kyaw Myint & *Thida*

**Department of Medical Research (Upper Myanmar)
**Department of Health*

Basic health staff (BHS) are frontline workers to carry out management of malaria at the community level. The objective of this study was to assess compliance level of BHS with national antimalarial treatment guideline (NAMTG). A cross-sectional analytic study was conducted from June 2006 to September 2007 at Singu, Thabeikkyin, Patheingyi, and Moegoke townships. A total of 144 BHS participated in this study. Among them, 71.5% had good-knowledge and 78.5% well complied with NAMTG. The rapid diagnostic test (RDT) allotment for each BHS was fifteen kits which ranged from zero to twenty-eight per month. Approximately two-third (68.8%) of BHS received enough RDT. Median ACT allotment for each BHS was two blisters which ranged from zero to fifteen per month. All BHS received enough ACT. In multivariate analysis, if BHS having good-knowledge were supplied with adequate RDT and ACT, they were very likely to become effective healthcare providers.

INTRODUCTION

In Myanmar, malaria is one of the priority diseases. It is a re-emerging public health problem due to climatic change, uncontrolled population migration, ecological changes, existence of multidrug-resistant *P. falciparum* parasite, appearance of insecticide resistant vector and change in behavior of vector [1]. About 70% of the populations are residing in malarious areas [2]. People residing in low or no risk areas may also contract malaria infection through migration on socio-economic reasons and easily develop severe form of disease leading to high mortality, unless they receive prompt appropriate effective treatment. The Government of the Union of Myanmar laid down the latest National Antimalarial Treatment Guidelines (NAMTG) in September 2002 with the aim to provide safe and rapidly effective antimalarial treatment to all patients with malaria and

to prevent the emergence and spread of drug resistance [3]. Out of total 325 townships, the implementation has been started as pilot project in 12 townships in May 2004. It was extended to 100 townships in 2006, including advocacy, training and distribution of Rapid Diagnostic Test (RDT) kits and antimalarials including Artemisinin-based Combination Therapy (ACT). In 2007, case management with ACT was practiced in all 325 townships [1]. The implementation of NAMTG is depending upon the health professionals, health workers, and all those involving in delivering the antimalarial treatments. Basic Health Staff (BHS) are frontline taskforces to combat malaria at the community level. Therefore, training on management of malaria according to latest NAMTG was given to BHS countrywide. Despite the training, the adherence of BHS on latest NAMTG has not been properly investigated. In this paper, the compliance of BHS with NAMTG was investigated.

MATERIALS AND METHODS

A cross-sectional analytic study was carried out from June 2006 to September 2007 at Singu, Thabeikkyin, Patheingyi, and Moegoke based on number of BHS trained and availability of RDT and ACT. It included all BHS giving antimalarial treatment: Health Assistant (HA); Township Health Nurse (THN); Lady Health Visitors (LHV); Public Health Supervisor I (PHSI); Midwives (MW); and Public Health Supervisor II (PHSII). A total of 144 BHS who has been working at least one year duration in recently assigned health facility, and who has not taken a long leave e.g. maternity leave, medical leave etc, within one year duration before this study were included in the study. Clients who were taking anti-malaria treatment at the respective facility at the time of survey or one week before survey were also interviewed. For data collection, knowledge of BHS on NAMTG was assessed using pre-tested self-administered questionnaire. Diagnosis and treatment practice were determined by patient records review. Check-list for healthcare facilities was used to assess the adequacy of diagnostic and treatment facilities. Face-to-face interview with clients was conducted using pre-tested semi-structured questionnaire to cross-check the responses of BHS regarding antimalarial treatment. .

RESULTS

Background characteristics of BHS

The mean age of BHS was 36.2 years. The youngest and oldest BHS were 22 and 59 years respectively. Most of the BHS were females (79.2%). Male to female ratio was 1:3.8. The studied BHS have been working as government servant for up to 10 years (37.5%), followed by 10- 19 years (34.0%), 20-29 years (21.5%) and over 30 years (6.9%). Regarding educational status, 41.7% were educated up to high school level while 46.5% were attending

university, and 11.8% were graduates. The majority (64.6%) was MWs. HA and PHSII were accounted for 10.4% each. Remaining 9.7% were LHVs. This study also included four PHSI (2.8%) and three THN (2.1%). A total of 144 BHS were studied (Table 1).

Table 1. Background characteristics of BHS

Variables	Frequency	Percent
<i>Age (Years)</i>		
20 – 29	58	40.3
30 – 39	33	22.9
40 – 49	30	20.8
50 – 59	23	16
Mean $\pm$ SD	36.2 $\pm$ 10.7	22 to 59
<i>Gender</i>		
Male	30	20.8
Female	114	79.2
<i>Service (years)</i>		
<10	54	37.5
10 – 19	49	34
20 – 29	31	21.5
30 – 39	10	6.9
Mean $\pm$ SD	13.6 $\pm$ 10.5	1 to 35
<i>Education</i>		
High school	60	41.7
University	67	46.5
Graduate	17	11.8
<i>Post</i>		
THN	3	2.1
HA	15	10.4
PHSI	4	2.8
LHV	14	9.7
MW	93	64.6
PHSII	15	10.4

THN = Township Health Nurse
 HA = Health Assistant
 PHSI = Public Health Supervisor I
 LHV = Lady Health Visitor
 MW = Midwife
 PHSII = Public Health Supervisor II

Training, supplies, knowledge and treatment practice of BHS

All BHS had been received training on NAMTG at least one time (Table 2). Approximately, half (49.3%) of the BHS has attended training for two times. Malaria Control Program made RDT and ACTs available down to Subcenter (SC) level where MWs are working. Therefore, distribution pattern of RDTs and ACTs was analyzed based on quotas for BHS at SCs (MWs, and PHSIIs). Only few BHS (10.4%) working at Township Health Department (THD), Urban Health Centers, and Maternal and Child Health Centre did not get RDT and ACT supply. The median

RDT allotment of each BHS was 15 kits per month. However, BHS needed about 17 RDT kits per month to confirm malaria among clinically suspected malaria (CSM) cases. RDT adequacy was calculated by subtracting number of CMS cases from RDT supply. Approximately, two-third (68.8%) of BHS received RDT adequately. The ACT allotment for each BHS was two blisters (one-adult course) per month with a range of zero to fifteen blisters. The median ACT usage per BHS was one blister per month with a range of zero to twelve blisters. The majority (86.1%) of BHS received ACT more than enough. Only 13.9% of BHS used all issued ACT every month (Table 2).

Knowledge level of BHS on NAMTG

A self-administered knowledge assessment questionnaire comprising of 25 variables was used to assess the knowledge level. Most of the BHS (94.4%) knew *Plasmodium falciparum* as the causal organism of severe and complicated malaria. *P. vivax* was regarded as benign form of non-falciparum malaria by 86.1% of BHS. However, only 36.1% knew all non-falciparum species including *P. vivax*, *P. ovalae*, and *P. malariae*. The incubation periods of *P. falciparum* and *P. vivax* could be written down by 74.3% and 64.6% of BHS respectively. Almost all BHS (96.5%) could enumerate 'signs and symptoms' of uncomplicated malaria and 84% knew at least three important signs and symptoms of severe and complicated malaria. Approximately three-fourth (71.5%) of BHS knew at least three correct differential diagnoses of uncomplicated malaria, and 79.2% knew three differential diagnoses of cerebral malaria. All BHS knew standard methods of malaria diagnosis as Blood for MP, and RDT. Almost all (98.6%) BHS could enumerate the drugs used in uncomplicated *P. falciparum* malaria. The treatment of mixed infection was correctly described by 75.7% of BHS. Regarding referral, 93.1% of BHS could mention at least three correct criteria and 89.6% of BHS has knowledge to

give a stat dose of injection artemether at the time of referral.

Table 2. Training, supplies, knowledge and treatment practice of BHS

Variables	Frequency	Percent
<i>No of training</i>		
1	33	22.9
2	71	49.3
3	25	17.4
4	15	10.4
Median (Range)	2	(1 to 4)
<i>RDT supply</i>		
< 10	51	35.4
15	32	22.2
16-20	15	10.4
21 -25	41	28.5
26 and above	5	3.5
Median (Range)	15	(0 to 28)
<i>No of CSM cases</i>		
< 10	43	29.9
15	28	19.4
16-20	22	15.3
21 -25	38	26.4
26 and above	13	9
Median (Range)	17	(0 to 55)
<i>RDT adequacy</i>		
Yes	99	68.8
No	45	31.3
<i>ACT supply</i>		
≥ 5	135	93.8
6 & above	9	6.3
Median (Range)	2	(0 to 15)
<i>ACT utilization</i>		
≥ 5	130	90.3
6 & above	14	9.7
Median (Range)	1	(0 to 12)
<i>ACT adequacy</i>		
Yes	124	86.1
No	20	13.9
<i>Knowledge level (Ordinal scale)</i>		
Poor	18	12.5
Fair	51	35.4
Good	75	52.1
<i>Knowledge level (Dichotomous scale)</i>		
Poor	41	28.5
Good	103	71.5
<i>Diagnosis practice</i>		
Clinically	5	3.5
Microscopy	8	5.6
RDT	131	91
<i>Treatment practice</i>		
Non-falciparum	8	5.6
ACT	136	94.4
<i>Practice level (Ordinal scale)</i>		
Poor	13	9
Fair	42	29.2
Good	89	61.8
<i>Practice level (Dichotomous scale)</i>		
Poor	31	21.5
Good	113	78.5

The correct dosage (number of tablets) and course of ACT containing 'artesunate plus mefloquine' for patients aged 15 years and

above, between 10 to 14 years, between 5 to 9 years, between 1 to 4 years, and under 1 year of age were mentioned by 92.4%, 89.6%, 91.7%, 77.8%, and 77.0% of total BHS respectively. The correct dosage and course of ACT containing “artesunate plus lumefantrine (Coartem)” for patients aged 15 years and above, between 10 to 14 years, between 5 to 9 years, between 1 to 4 years, and under 1 year of age were written down by 98.6%, 97.2%, 93.0%, 97.9, and 100% of BHS respectively. The anti-malaria treatment for pregnant mother was correctly described by 64.4% of BHS.

The mean knowledge score of 32.5 and SD of 3.2 were used as parameters. BHS who achieved knowledge score below 29.3 (Mean-SD) were regarded as poor knowledge, from 29.4 to 35.6 (Mean $\pm$ SD) as fair knowledge, and more than 35.7 (Mean + SD) as good-knowledge on NAMTG.

The results revealed the proportions of BHS who had good, fair, and poor knowledge on NAMTG were 52.1%, 35.4%, and 12.5% respectively. Data were again categorized into dichotomous scale by using mean knowledge score as cut-off point. Results found that 71.5% had good-knowledge and 28.5% had poor-knowledge on NAMTG.

Diagnosis and treatment practice

RDT was used to confirm malaria among CSM cases by 91% of BHS. Microscopic diagnosis was done by eight BHS working at Rural Health Centers (RHCs). Only five BHS clinically diagnosed CSM case. BHS treated all confirmed *P. falciparum* malaria patients (n=136) with ACT. Non-falciparum malaria treatment was given to clinically diagnosed patients (n=8).

Cross check on methods of diagnosis, treatment, and treatment completion

BHS responses regarding diagnosis, treatment category, and treatment completeness were cross-checked with patients' responses using Kappa statistic and highly significant agreements were found ($p < 0.001$). An

absolute agreement between BHS and patients was found on 'clinical diagnosis' (n=5). However, two out of eight patients said they were clinically diagnosed while BHS said they used microscopy. Among 131 patients whom diagnosed by RDT according to BHS words, 16 patients said they were diagnosed clinically, and 2 patients said they were diagnosed by microscopy (Kappa value= 0.455, $p < 0.000$). Absolute agreements on giving ACT to RDT positive cases and giving non-falciparum treatment to RDT negative cases were found between BHS and patients' response.

Compliance of BHS on NAMTG

Practice scores were given for the number of *P. falciparum* malaria patients diagnosed by RDT or microscopy (during last month), the number of patients treated using a complete course of ACT(during last month), and the number of patients referred to hospital (during last year). The mean score of 6.3 and SD of 4.3 were used as parameters. BHS who achieved practice score below 2.0 (Mean – SD) were regarded as poorly-complied, between 2.0 to 10.6 (Mean $\pm$ SD) as fairly-complied, and more than 10.6 (Mean+ SD) as well-complied with NAMTG. The results revealed the proportions of BHS who poorly-complied, fairly-complied, and well-complied with NAMTG were 9.0%, 29.2%, and 61.8% respectively. Data were again categorized into dichotomous scale by using mean practice score as cutoff point. The results determined the proportions of BHS who 'well-complied' and 'poorly-complied' with NAMTG were 78.5% and 21.5% respectively.

Factors responsible for compliance of BHS on NAMTG

In bivariate analysis (crude analysis): male BHS were 4.8 times more complied with NAMTG compared to females. University attending or graduate BHS were 1.7 times more likely to adhere on NAMTG in comparison to less educated BHS. BHS

working at THD or RHCs were 4.3 times more complaint on NAMTG compared to those working at more peripheral levels. If BHS thoroughly knew the essence of NAMTG, those were 16.7 times more likely to be well-complied compared to BHS with poor knowledge. If BHS were supplied with RDT and ACT adequately, they were 15.2 times more likely to be efficient in malaria treatment compared to those receiving insufficient supplies.

In multiple logistic regression analysis: the combined effect of gender, education, post, knowledge, and ACT supply status was highly significant on likelihood of BHS compliance with NAMTG ($R^2=0.35$, $p<0.000$). If BHS with good knowledge on NAMTG (Adjusted OR =12.2) were supplied with adequate RDT and ACT (Adjusted OR=14.5), they were very likely to be effective healthcare providers in management of malaria according to NAMTG (Table 3).

DISCUSSION

The Government of the Union of Myanmar adopted a new anti-malaria treatment policy and laid down the latest NAMTG in September 2002, which superseded the previous national anti-malarial drug guidelines to provide safe and rapidly effective antimalarial treatment to all patients with malaria and to prevent the emergence and spread of drug resistance [3]. The change of policy was a long and complex process involving key stakeholders and decision-makers [4] as well as frontline workers at the community level. This study assessed the level of compliance whether BHS has already known and accepted the new guideline or defiant somehow four years after implementation. In Tanzania, a study was conducted how communities in a Tanzanian rural district adopted the new policy less than a year after the implementation of a new first line antimalarial drug [5].

Table 3. Factors responsible for compliance of BHS on NAMTG

Factors	Poorly complied (no.)	Well complied (no.)	Crude OR (95%CI)	P	Adjusted OR (95%CI)	P value
Age						
Young <35 Yrs	19	65	1.2	0.706*	NA	-
Old ≥35 Yrs	12	48	(0.5- 2.9)			
Service						
<13Yrs	18	62	1.1	0.751*	NA	-
≥13Yrs	13	51	(0.5 -2.8)			
Gender						
Female	28	85	4.8	0.026**	1.1	0.082
Male	2	29	(1.1- 3.5)		0.9- 2.9	
Education						
≤ high school	16	44	1.7	0.020*	2.4	0.131
≥university	15	69	(1.2- 4.0)		0.8- 7.4	
Post						
LHV,MW, PHSII	17	25	4.3	0.000*	9.1	0.052
THN, HA, PHSI	14	88	(1.7- 10.7)		0.9- 4.7	
Training						
1 time	6	27	1.3	0.594*	NA	-
≥2 times	25	86	(0.5- 4.3)			
Knowledge						
Poor	15	6	16.7	0.000*	12.2	0
Good	16	107	(5.1-57.5)		10.2-18.9	
Supply						
Not-adequate	23	8	15.2	0.009*	14.5	0.001
Adequate	18	95	(5.4-44.2)		2.9-65.1	

* Pearson's chi-square, ** Fisher's Exact test

THN = Township Health Nurse

HA = Health Assistant

PHSI = Public Health Supervisor I

LHV = Lady Health Visitor

MW = Midwife

PHSII = Public Health Supervisor II

Results of this study show that majority of BHS have good-knowledge and well-complied with NAMTG. Similar finding was reported in Cambodia study, where village malaria workers and malaria outreach team workers generally prescribed ACT according to blood test results done by RDT [6]. In our study, good-knowledge and supplementation of RDT and ACT were determined as significant factors influencing well-compliance with NAMTG. Evidence on improvement in basic health

services through supplying training and diagnostic equipments was reported in Myanmar. Japan International Cooperation Agency implemented a 5-year long bilateral technical cooperation project. The project conducted a series of refresher trainings for BHS of project areas to improve the basic health services for 3 years (2001-2003) and found improvement of services at township level hospitals through supply of microscope and microscopic training on leprosy, malaria, and TB [7]. A study conducted in the remote malarious north western Thai-Myanmar border reported the early detection of malaria by trained village volunteers, using rapid diagnostic tests and treatment with mefloquineartesunate was feasible and reduced the morbidity and mortality of multidrug-resistant *P. falciparum* [8].

RDT and ACT were distributed from THD to RHCs on monthly basis. Number of RDT and ACT to be distributed was estimated based on previous month utilization. HA is responsible for distributing RDT and ACT from RHC sub-stock to SCs. MWs need to return 'used RDT strips' and 'used ACT blisters' to confirm the actual utilization. Majority of LHV and BHS at SCs were receiving an adequate amount of RDTs and ACTs without interruption. However, in malaria season (June–October) some highly endemic areas e.g. Thabeikkyin, the case load overshoot the supply. BHS used up almost all issued RDTs but they could not use all ACTs. The reason was they were unable to wise enough to differentiate malaria and non-malaria fevers. As a result, yield of RDT to identify the malaria cases was reduced. Although they used almost all of RDTs, they didn't get enough falciparum positive malaria cases to use ACT. ACTs were left in their hands. BHS revealed that they used to give non-falciparum treatment to RDT negative but highly suspicious malaria cases.

Technical and operational problems on using RDT and ACT were reported from many developing countries. In Cambodia, after switching antimalarial drug policy to

use RDT and ACTs, one study reported that the coverage rates of appropriate diagnosis and treatment of malaria were very low (only 11% of adults and 2% of children under five years) and authors concluded that it was due to the fragmented nature of Cambodia's health system in remote areas [6]. A study in Thailand reported that changes in diagnostic tools from microscopy to a rapid test (that detects *P. falciparum* only) underestimated the number of *P. vivax* cases [8]. A study in sub-Saharan Africa suggested that a reliable supply of ACT to facilities where most patients are seeking treatment for malaria-like symptoms was essential for proper policy implementation [9].

Basing on the townships of our study we concluded that provision of effective on-job trainings for healthcare providers down to grass-root levels supported by adequate and uninterrupted diagnostic and treatment facilities (RDT and ACT) will ensure the proper implementation of the national anti-malarial treatment policy at the community level.

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