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**Synthesis of Health Systems Research under the framework of
Health Research Programme: a decade work of
Department of Medical Research, Lower Myanmar (2000-2009)**

*Le Le Win, Saw Saw, Yin Thet Nu Oo, Khin Sandar Oo,
Myo Khin, Thandar Min & Soe Moe Myat*

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Prime purpose of Health Systems Research (HSR) is “to help improve health of people through improvement not only of conventional health services but also of other services that have a bearing on health”. With this aim, divisions within Department of Medical Research (Lower Myanmar) (DMR-LM) have been conducting HSR-related projects since 1986. However, there is a gap of information on linkage between these research projects and Health Research Programme (HRP) of National Health Plan (NHP) and their utilization. Thus to fulfil this gap, research summaries from Annual Reports of DMR-LM from 2000 to 2009 were reviewed and categorized into 7 projects under HRP using content analysis. During this decade, 16 research divisions and one research unit of DMR-LM conducted 160 HSR-related projects. About 61% of the projects were conducted by social science research divisions. Number of projects conducted over the years had increased due to involvement of clinical and laboratory-based research divisions in HSR projects particularly in 2004. Of them, about half were communicable diseases followed by reproductive health, non-communicable diseases and environmental health. Majority was health systems-related projects under HRP (83.8%), of which 67.9% were conducted by social science-related divisions. It could be due to nature of work of the respective divisions. Collectively, most findings were academically utilised (85.6%), and about 13% were utilized by project managers (n=21) for various purposes. The findings indicate that there is still a need to promote result utilization by service departments by involving both researchers and service managers.

INTRODUCTION

World Health Organization (WHO) had defined a health system as ‘consists of all organizations, people and actions whose primary intent is to promote, restore or maintain health’ [1]. To improve health systems, three research domains including operational, implementation and health system play an important role in conducting the research or projects [2]. Of these three domains, Health Systems Research (HSR) is defined broadly as the production of new knowledge to improve how societies organize themselves to achieve health goals [3]. Prime purpose of HSR is to help improve health of people through improvement not

only of conventional health services but also of other services that have a bearing on health. Accordingly, divisions within the Department of Medical Research (Lower Myanmar) (DMR-LM) have been conducting HSR-related projects since 1986. More or less, these researches were related to 7 projects under Health Research Programme (HRP) of National Health Plan (NHP) 2006-2011, namely, communicable diseases (CD), non-communicable diseases (NCD), health systems, environmental health (EH), traditional medicine, academic and technology development and capacity strengthening. However, there are limited information on the linkage between these research projects and HRP and their

utilization. With this aim, this review study was conducted to determine types of HSR-related projects under the framework of HRP conducted by divisions within DMR-LM from 2000 to 2009 and the degree of research utility.

MATERIALS AND METHODS

For consistency of source of information, Annual Reports of DMR-LM from 2000 to 2009 were used [5]. All HSR-related projects in these reports were analysed. Based on the year of reporting the research findings, the summaries of the research findings were reviewed and categorized into seven projects under HRP of NHP using content analysis. Regarding the situation of utilization of research findings, scientists from the respective divisions of DMR-LM were requested to fill in a form on research utilization.

RESULTS AND DISCUSSION

During a 10-year period, 16 of 22 research divisions and one of seven research units of DMR-LM conducted 160 HSR-related projects.

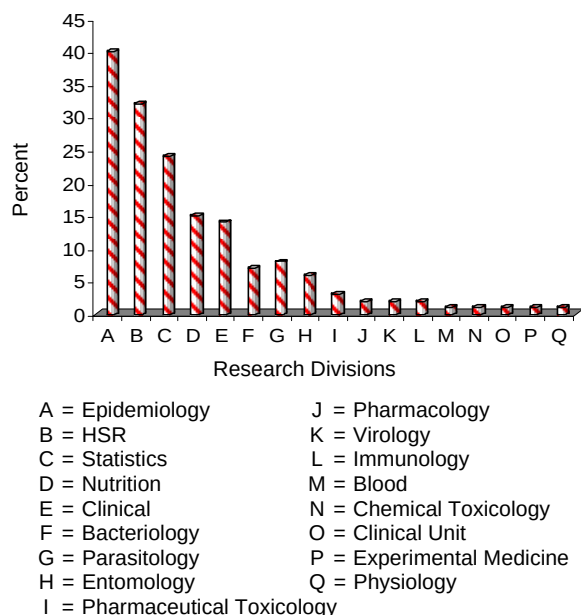


Fig. 1. HSR-related projects conducted at DMR-LM during 2000-2009 by research divisions

Social science-related research divisions (i.e., Epidemiology, Health Systems Research

and Medical Statistics research divisions) conducted 60.6% of the projects and clinical and laboratory-based research divisions conducted 39.4% of the projects, respectively (Fig. 1).

Yearly HSR-related projects conducted by divisions

During the study period, while number of projects conducted over the years had increased, number of projects conducted by social science-related divisions had decreased between 2001 and 2006, particularly in 2004. It could be due to increased involvement of clinical and laboratory-based research divisions in HSR projects, more collaboration among divisions with different disciplines of DMR-LM and most researchers from social science-related research divisions were out of office for further studies during 2001 and 2006 (Fig. 2).

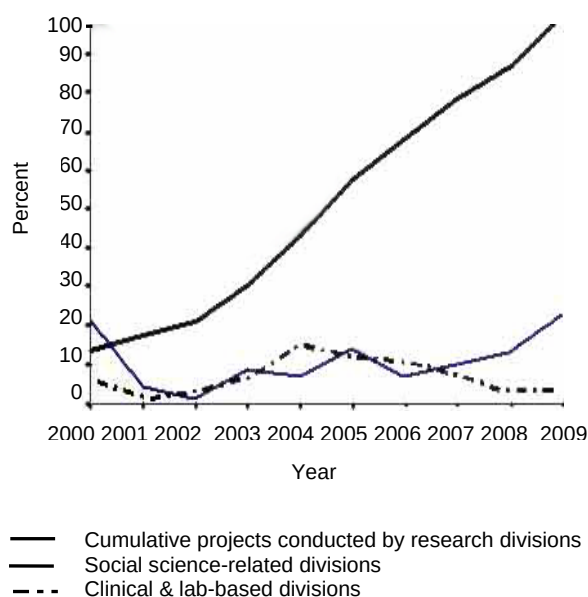


Fig. 2. Yearly HSR-related projects conducted by divisions from 2000 to 2009

Types of HSR-related researches conducted by divisions

During this decade, social science-related research divisions conducted 97 projects (60.6%) and clinical and laboratory-based research divisions conducted 63 projects (39.4%), respectively (Table 1).

Table 1. Type of disease-related projects by Health Research Programme conducted by research divisions

Project under Health Research Programme	Type of disease	Research Division		Total no. (%) [*]
		Social science-related divisions	Clinical & lab-based divisions	
Health systems	Communicable diseases	51	21	72
	Reproductive health	23	1	24
	NCD	0	3	3
	Environmental health	0	2	3
	Other	16	16	32
Sub total		91	43	134(83.8)
Non-communicable disease	Non-communicable diseases	1	6	7
	Reproductive health	0	1	1
	Other	2	3	5
Sub total		2	10	13(8.1)
Communicable disease	Communicable diseases	2	7	9
Sub total		2	7	9(5.6)
Environmental health	Environmental health	0	3	3
Sub total		0	3	3(1.9)
Other	Other	1	0	1
Sub total		1	0	1(0.6)
Total (%) ^{**}		97 (60.6)	63 (39.4)	160

*Column percent **Row percent

By type of disease-related research, about half (50.6%) were communicable diseases including AIDS, TB, malaria, leprosy, ARI, DHF and HCV. Next came reproductive health (15.6%) followed by non-communicable diseases (6.3%) and environmental health (3.8%). About one-fourth were other research areas such as smoking practice, elderly, research implementation, cost study, snake bite, minor ailments, vital statistics, dietary pattern, food behaviour, iron status, health information, etc. Both social science-related research divisions and clinical and laboratory-based divisions focused more on communicable diseases (53/97=54.6% and 28/63=44.4%, respectively).

With respect to the projects under HRP, the majority of researches were concerned with health systems (83.8%), and these were relating to treatment seeking behaviours, establishment of community-based surveillance system for women cancer, inter-

vention studies for sustainable dengue control and malaria prevention. Of which, 67.9% were done by social science-related research divisions. Clinical and laboratory-based research divisions also emphasised on NCD, communicable diseases and environmental health relating to HRP. Environmental health-related studies were done by Pharmaceutical Toxicology, Chemical Toxicology, Entomology and Pharmacology research divisions.

Utilization of research findings

Collectively, most findings were academically utilised by either one of the following channels (n=137, 85.6%): presentation at Myanmar Health Research Congress, reporting to Department of Health and international agencies, dissemination at seminars, thesis and publications in local and international journals. Of which, reporting and presentation at the congress were the commonest. The situation of result finding of 2 researches was not known.

About 13% of the research findings were utilized by project managers (n=21) as a citation or reference, guidance for patient treatment and guidelines for health providers of the respective programme and general practitioners. Among them, 11 and 10 researches were conducted by social science-related research divisions and clinical and laboratory-based research divisions, respectively. Since the findings were based on the personal contact of the respective divisions of DMR-LM, we could not know the real utilization status from the service department's aspect. It is assumed to be larger than this extent.

It was observed that most of the research results utilized by the service managers were communicable diseases (62%), which were among the priority diseases ranked in NHP 2006-2011 such as TB, malaria and leprosy (Fig. 3). In addition, with regards to HRP, while research findings relating to health systems were mostly used by the service departments, other types such as communicable diseases, non-communicable diseases and environmental health were

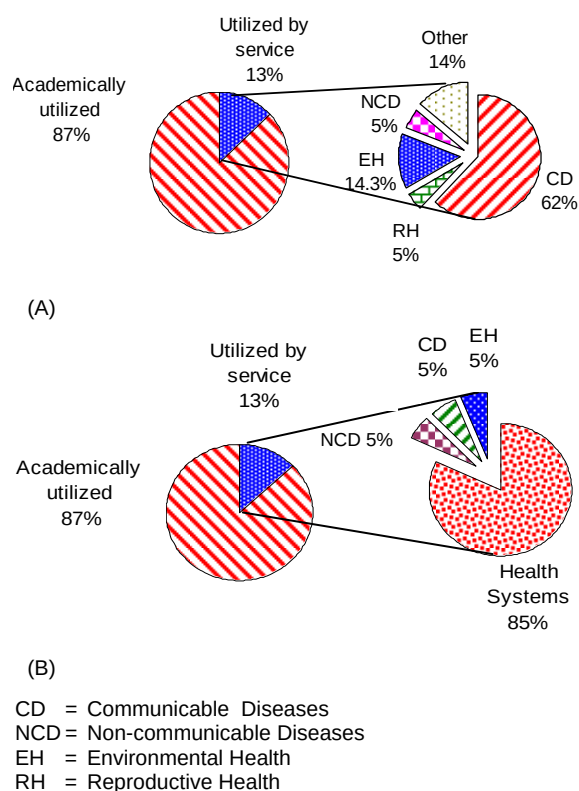


Fig. 3. Situation of research findings utilization by (A) type of disease-related projects and (B) Health Research Program

the least (5% each). Through personal communication, it was also noted that service managers participated in those researches from the beginning of protocol development.

Conclusion

During the 10-year study period, 160 HSR-related projects were conducted by more than half of the research divisions of DMR-LM. However, some of the research findings of ongoing projects were not documented in Annual Reports; in reality, number of the projects could be larger than this. However, it was observed that by effective collaboration among divisions of DMR-LM, HSR-related projects of DMR-LM contributed to health development of the country. However, it still needs to promote research areas relating to Environmental Health. It was noted that both types of research divisions of DMR-LM conducted the research, of which the results were used by the service

departments. The research findings were used by the service departments for various purposes. However, the extent of result utilization was not fully satisfactory. The situation inclines to the view of Remme and his group [2] that recognition of role of research in improving health system and health care delivery had increased and concurrently, result utility which is useful and serviceable, should also be taken into consideration.

Although the real situation of research utilization was not known, the findings indicate that there is still a need to promote result utilization by service departments. Thus, not only the researchers need to be encouraged to involve service managers in implementation of research but also the service managers need to be encouraged to seek information for utilization through various channels. To achieve this, the following ways and means could be helpful to enhance the utilization of research findings:

- Preparing research findings in brief or executive summary for the service managers
- Making this summary reach to service managers, and
- Creating a situation for sharing the findings among researchers and the service managers

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**Acute and sub-acute toxicity studies of Traditional Medicine
Formulation Number 28 (*Thetyinnkalat Hsay*) on rat**

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The purpose of this study is to perform standardization and to find out the safety profile of Traditional Medicine Formulation Number 28 (*Thetyinnkalat Hsay*) on laboratory rat model. In an acute toxicity study, TMF 28 was administered orally at three different doses 9.2 g/kg body weight (80 times human dose), 4.6 g/kg body weight (40 times human dose), and 2.3 g/kg body weight (20 times human dose), and distilled water to control group by using Lichfield and Wilcoxon Method. The results indicated there were no toxic symptoms up to the dose level of 9.2 g/kg per orally (p.o). In sub-acute toxicity study, this drug was tested at three doses of 2 g/kg body weight, 1 g/kg body weight and 0.5 g/kg body weight p.o once daily for 90 days. The animals were sacrificed on the 91st day and various blood biochemical parameters, haematological, and histopathological examinations were done. Sub-acute toxicity test showed that there was no decrease in body weight except the dose of 2 g/kg body weight. No significant changes in weight of the internal organs such as heart, liver, lung, kidney, spleen, stomach and intestine were found, when compared with the control group. No significant changes in liver and kidney functions tests, and haematological parameters were observed when compared with the control group. Mild congestion of capillaries and blood vessels were observed in heart, lung and liver of some rats. These findings suggested that dosage prescription of TMF 28 should be carefully selected on conducting clinical trial to ensure the maximal safety of this drug.

INTRODUCTION

Many traditional healing herbs and plant parts have been shown to have medicinal value, especially in the rural areas. These can be used to prevent, alleviate or cure several human diseases. The WHO estimates that more than 80% of the world's population rely either solely or largely on traditional remedies for health care. Traditional Medicine Formulation Number 28 (TMF No. 28), *Thetyinnkalat Hsay*, anti-diabetic drug has been used for long time but its sub-acute toxicity (long term) has not been studied. Human dose of TMF No. 28 applied in our study is 270 mg/tablet×7 tablets×3 times per day which is approximately equivalent to 115 mg/kg body weight. The present study aims to conduct the standardi-

zation and safety (acute and sub-acute toxicity) of TMF No. 28 after three months administration to laboratory animals. The outcome of the study will be utilized as safe and recommended dosage for patients with diabetes mellitus.

MATERIALS AND METHODS

Ingredients

Scientific name	Myanmar name	Amount in 100.4 g/100 g
<i>Croton oblongifolius</i> (root)	Thetyinnngyee	50.0
<i>Capparis sepiaria</i> (bark)	Hsoogauknet	5.6
<i>Crataeva religiosa</i> (bark)	Kadet	5.6
<i>Piper nigrum</i> (fruit)	Ngayokkoun	5.6
<i>Osyris wightiana</i> (fruit)	Zaunggyan	5.6
<i>Piper longum</i> (fruit)	Peikchinn	5.6
<i>Sapium</i> sp.(stem)	Awle	5.6
<i>Zingiber officinale</i> (root)	Gyin	5.6
Unidentified (root)	Hsayple	5.6
Roasted salt	Hsahlaw	5.6

Standardization of TMF No. 28

TMF No. 28 was standardized by general analytical and pharmacopoeial methods [1, 2, 3].

Phytochemical test for types of compounds

Preliminary detection of phytochemical compounds present in TMF No. 28 was carried out according to the general methods of Harborne [4] and Unani Formulation [5].

Acute toxicity study

Four groups of 10 adult mice (ddy strain) of both sexes each weighing 25 ± 5 g were kept in cages of five each. The mice were treated with three different doses of 9.2 g/kg (80 times the effective dose), 4.6 g/kg (40 times the effective dose) and, 2.3 g/kg (20 times the effective dose) of TMF No. 28 dissolved in water by using feeding nozzle. They were observed daily for two weeks. The lethality (LD_{50}) was calculated using the method of Lichfield and Wilcoxon [6].

Sub-acute toxicity study

For sub-acute toxicity, four groups of six albino rats (Wistar strain), each weighing 225 ± 25 g of both sexes were kept in separate cages. The rats were treated with 2 g/kg, 1 g/kg and 0.5 g/kg of TMF No. 28 using feeding nozzle for three months [7, 8, 9]. They were closely observed for three months. After that they were held another 24 hours before sacrifice. Then, the animals were sacrificed, the blood and the internal organs were collected.

Laboratory investigations

After three months of drug administration, renal and liver function tests (aspartate transaminase (AST, GOT), alanine transaminase (ALT, GPT), alkaline phosphatase (ALP) and total bilirubin) haematological tests (complete picture) and histopathological examination were performed on heart, liver, lung, kidney, spleen, stomach and small intestine.

Statistical analysis

Comparative data of control and experimental animal test groups investigated in

sub-acute toxicity test were analyzed by using Student's 't' test.

RESULTS

The quality control data of TMF No. 28 are shown in Table 1 and 2.

Table 1. Pharmacopoeial specifications and quality criteria of TMF No. 28

Test parameter	Results
Appearance	Pale white color tablet
Average weight	0.27 g/tab
Uniformity of weight of tablet	Comply with Indian and British pharmacopoeias
Disintegration time (limit not more than 15 mins)	1 min

Table 2. Physicochemical data of TMF No. 28

Test parameter	Results
<i>Organoleptic characteristics</i>	
Appearance	Tablet
Colour	Pale white
Odour	Characteristic
Taste	Hot - bitter
Loss on drying at 105 °C	8.28%
<i>Ash values</i>	
Total ash (%w/w)	9.8-10.7%
Water soluble ash (%w/w)	8-8.7%
Acid insoluble ash (%w/w)	0.6-0.7 %
<i>Extractable matters</i>	
Water soluble matters (hot extract)	24.48%
Ethanol (96%) soluble matters (cold maceration)	12.54%
Bitterness value	12.2 unit/g
Foaming index (height 16 cm, diameter 16 mm)	<100

Phytochemical test showed flavonoids, glycoside, carbohydrate, saponins, tannins, triterpene and steroids were present but alkaloids, resins, phenols and protein were not present in this TMF No. 28.

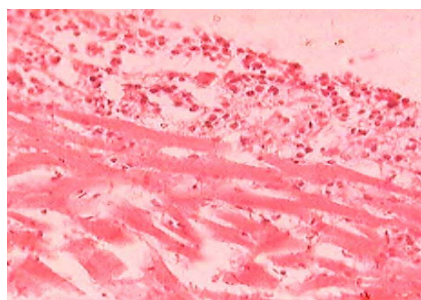
According to acute toxicity test, none of the mice died in three different doses (2.3 g/kg, 4.6 g/kg and 9.2 g/kg). Thus, LD_{50} must be above the dose of 9.2 g/kg.

In sub-acute toxicity test, rats treated with various doses (0.5 g/kg, 1 g/kg and 2 g/kg) had a progressive weight gain except the dose of 2 g/kg body weight. No significant changes in weight of the internal organs such as heart, liver, lung, kidney, spleen, stomach and intestine were observed in all groups.

No significant difference in biochemical parameters such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilirubin. Haematological parameters (complete picture) were observed between tests and control group of laboratory animals.

Histopathological findings of internal organ of rats

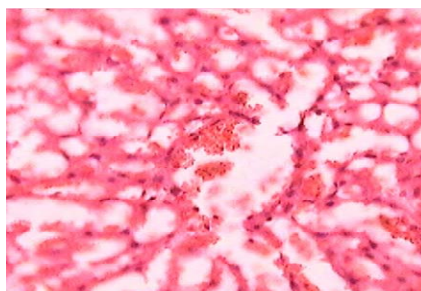
Mild congestion of capillaries and blood vessels were observed in heart, lung and liver of some rats.



Heart
2 g/kg
Infiltration of subepicardial by lymphocytes and plasma cells



Lung
2 g/kg
Congestion of alveoli capillaries with haemorrhages



Liver
1 g/kg
Mild congestion of central veins and sinusoids

DISCUSSION

This study brings out a safe traditional medicine which will hopefully be useful for the treatment of diabetes. In our study, the raw materials of TMF No. 28 come from various sources with different geographical background. So, physicochemical data cannot be the same as that mentioned in the previous study conducted in DMR (LM) in

1984. There were no toxic symptoms up to the dose level of 9.2 g/kg (80 times the effective dose). After three months of drug administration, there was no decrease in body weight except the dose of 2 g/kg body weight. No significant changes in weight of the internal organs such as heart, liver, lung, kidney, spleen, stomach and intestine were found, when compared with the control group. No significant changes in liver and kidney functions tests, and haematological parameters were observed when compared with the control group. Mild congestion of capillaries and blood vessels were observed in heart, lungs and liver of some rats with 80 times higher than human dose assigned for this particular study, i.e., 2 g/kg body weight. Therefore, dosage titration in humans may avoid such kind of histological damage. These findings suggested that dosage prescription of TMF No. 28 should be carefully selected on conducting clinical trial to ensure the maximal safety of this drug.

Conclusion

It can be concluded that dosage of TMF No. 28 should be carefully selected on conducting clinical trial to ensure the maximal safety.

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**Dietary habit of type 2 diabetes mellitus patients
attending diabetes clinics in Yangon**

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To find out the dietary habit of type 2 diabetes mellitus patients, a descriptive study was carried out in one hundred and one type 2 diabetes mellitus patients of both sexes attending the diabetic clinic of North Okalapa General Hospital and a specialist clinic of one private hospital in Yangon. The diagnosis had been confirmed for less than two months when they participated in the study. Thirty-nine men (38.6%) and 62 women (61.4%) were interviewed by using the food frequencies questionnaire for previous dietary habit (before they had been known as the diabetics) and structured questionnaire for knowledge on dietary habit of type 2 diabetes mellitus. The combined 24-hour recalled method and food diary method were used to assess the food and nutrient intakes of current situation (after the diagnosis). Mean energy intake (kcal) of 1932.7 ± 562.3 and 1428.3 ± 251.1 were found in 20-59 years and ≥ 60 years old men, respectively, and 1593.3 ± 386.9 and 1704.2 ± 420.6 were found in 20-59 years and ≥ 60 years old women, respectively. Percent contribution of carbohydrate to total calories of men and women was around 65%. Percent contribution of protein to total calories was around 10% and fat was around 20-24%. Food taboos were present among diabetes patients such as eating of rice with low quality (eg. *Nga-sein*) and cooked rice with surplus water that had been drained off. Twelve percent of subjects ate Basmati rice (so called *see-cho* rice). Regarding nutrition knowledge of diabetes, most of the patients (91.2%) avoided sweet foods but 12 (17.6%) and 10 (14.7%) patients answered they consumed more vegetables and reduced fat intake. So, nutrition education is still needed to improve dietary habit of diabetes patients.

INTRODUCTION

Diabetes mellitus is a chronic disease which requires continuing medical care to prevent acute complications and to reduce the risk of long-term complications. It requires a multiple approach that would include medical nutrition therapy, weight reduction and use of drugs when indicated [1]. From the study done in 2003, it was found that prevalence of diabetes among 4613 people aged 20 years and above in Yangon Division was 11.9% (95% CI, 10.1 to 13.4%). Urban prevalence was 13.9% and rural prevalence was 7.3% [2].

Nutrition remains a cornerstone in the management of diabetes [3]. Meal planning

is a central element of nutrition therapy, and food selection is the first step in meal planning. Food selection affects the nutrient and energy content of the diet and the metabolic control of blood glucose, lipids, and body weight of people with diabetes. The nutrition information on food labels can guide food selection. The overall dietary pattern may affect health more than individual foods and nutrients do. Recent dietary intervention studies have indicated that interventions focused on dietary patterns can decrease blood pressure and reduce cardiovascular complications [4].

Diet and exercise are considered important components of the treatment strategy for adults with type 2 diabetes. Appropriate use

of diet and exercise can improve insulin sensitivity and glycemic control and decrease the need for oral medications or insulin [5]. In a survey of more than 2,000 adults with diabetes, the most frequently reported barriers in diabetes self-management were adherence to diet and exercise [6]. Only 60% of individuals with diabetes in the National Health Interview Survey reported that they followed a diabetic diet [7], and several non-U.S. studies have reported that actual nutrient intake among individuals with diabetes may be suboptimal [8].

Nutritional management is a cost-effective approach to health care. Nutrition reduces the cost, especially those associated with specific medications, morbidity, length of hospital stay and mortality. This can be achieved through adequate, practical and effective nutrition intervention based on scientific fact. Data on dietary patterns for type 2 diabetes are sparse. Therefore, this study aims to identify previous dietary habit of type 2 diabetes mellitus patients (before they had been known as diabetics) and to assess their nutrients intake after the diagnosis.

MATERIALS AND METHODS

It was a cross-sectional descriptive study and was conducted in the diabetic clinic of North Okkalapa General Hospital and a specialist clinic of the private hospital in Yangon. One hundred and one newly diagnosed type 2 diabetes mellitus patients attending the above-mentioned clinics participated in the study.

The diagnosis had been confirmed for less than two months with the standard procedures and by the physicians. They were interviewed by structured questionnaire containing socio-demographic background, knowledge on dietary habit and food frequency questionnaires for previous dietary habit (before the diagnosis). The combined 24-hour recalled and food diary methods

were used to assess the current nutrient intakes of the patients.

Data entry and analysis were done with Epi info 6 software after checking and validation.

RESULTS

Background characteristics

Of the total of 101 type 2 diabetes mellitus patients, 39 (38.6%) were males and 62 (61.4%) were females. The age range was 27-87 years and the mean $\pm$ SD was 49.6 $\pm$ 10.8 years. Eighty-four percent of subjects were interviewed at the specialist clinic and 16% were interviewed at the diabetic clinic of North Okkalapa General Hospital. About fourteen percent of subjects had education with less than primary school level and only 26.7 % was graduated. Their median monthly income was 80000 kyats, minimum 5,000 kyats and maximum was 10,000,000 kyats.

Previous dietary habit

Daily consumption of fish, chicken/duck, beef/mutton, pork and egg were by 9.9%, 3%, 1%, 2% and 4% of subjects, respectively. Most of the subjects (65.3%) ate fish 4-5 days per week. More than half of the subjects (57%) consumed vegetables 6-7 days per week.

Table 1 shows the consumption of beverages, sweet foods, and oily food of subjects before they have been known as diabetics. According to habits of consumption of beverages, 58 (74.4%) and 10 (14.1%) of subjects daily drink coffee/ tea and cold drinks/ juice, respectively. According to consumption of sweet food, 18%, 6.7%, 9.2% and 4.8% of subjects daily ate jaggery/ brown slab-sugar (*kjandaga*), chocolate, snacks made with sugar and snacks made with jaggery/ *kjandaga*, respectively. Regarding oily foods, 21.3%, 20.3% and 3.2% of subjects consumed deep fried food, food made with coconut milk and rice cooked with butter one day per week, respectively.

Table 1. Consumption of beverages, sweet food, and oily food of study subjects before the diagnosis of diabetes mellitus

Food items	Habits (%)	Number of day per week	Frequency No (%)
Beverages	Drinking coffee/ tea	6-7	58 (74.4)
	Yes 78 (77.2)	4-5	4 (5.1)
	No 23 (22.8)	2-3	6 (7.7)
		1	4 (5.1)
		<1	6 (7.8)
	Drinking cold drinks/ Juice	6-7	10 (14.1)
		4-5	7 (9.9)
	Yes 71 (70.3)	2-3	11 (15.5)
	No 30 (29.7)	1	13 (18.3)
		<1	30 (42.3)
Sweet foods	Eating jaggery/ brown slab-sugar	6-7	9 (18)
	Yes 50 (49.5)	4-5	5 (10)
	No 51 (50.5)	2-3	6 (12)
		1	11 (22)
		<1	19 (38)
	Eating snacks made with jaggery/ brown slab-sugar	6-7	3 (4.8)
	Yes 63 (62.4)	2-3	13 (20.6)
	No 38 (37.6)	1	12 (19)
		<1	35 (55.5)
	Eating creamy cake/ bread	4-5	1 (1.7)
Oily foods	Yes 58 (57.4)	2-3	13 (22.4)
	No 43 (42.6)	1	11 (19)
		<1	33 (56.9)
	Eating deep fried food	6-7	4 (4.5)
	Yes 89 (88.1)	4-5	3 (3.4)
	No 12 (11.9)	2-3	29 (32.6)
		1	19 (21.3)
		<1	34 (38.29)
	Eating food made with coconut milk	4-5	2 (3.1)
	Yes 64 (63.4)	2-3	13 (20.3)
	No 37 (36.6)	1	13 (20.3)
		<1	36 (56.3)
	Eating rice cooked with butter	1	2 (3.2)
	Yes 63 (62.4)	<1	61 (96.8)
	No 38 (37.6)		

Knowledge on healthy lifestyle of type 2 diabetes mellitus patient

When the subjects were asked whether they had known about the healthy life style of type 2 diabetes mellitus patient, 67 (66.3%) answered "yes". Among them, most of the subjects (91.2%) answered to reduce intake of sweetened foods. Table 2 shows knowledge on healthy lifestyle of type 2 diabetes mellitus patient.

When asking about nutrition education for diabetes, 67 subjects (66.3%) answered that they got nutrition education about diabetes.

Table 2. Knowledge on healthy lifestyle of type 2 diabetes mellitus patient

Knowledge	Number	Percent
Knows to reduce intake of sweetened food	62	91.2
Knows to consume more vegetables	12	17.6
Knows to reduce fat intake	10	14.7
Knows to avoid sweet fruits	9	13.2
Knows to reduce rice intake	6	8.8
Knows to do exercise	6	8.8
Knows to reduce salty foods	3	4.4
Knows to reduce food intake	2	2.9

Among them, 27% and 21% of subjects answered they got nutrition education about diabetes from physicians and general practitioners, respectively. Another 21% got nutrition education from pamphlets. About 10% of subjects got nutrition education for diabetes from friends. Only one subject answered she got nutrition education from television.

Eating habit of type 2 diabetes mellitus patients after diabetes

Only 30 subjects answered that they ate special diet (changing food habit after onset of diabetes). Among them, 36% answered they reduced rice intake, 32% ate rice with low quality (*San-kyan*) like *Nga-sein* and 24% ate rice cooked with discarding surplus water. Twelve percent of subjects ate Basmati rice (so called *see-cho* rice).

Current nutrient intake of study patients

Mean energy and nutrient intakes, percent Recommended Dietary Allowances (RDA) of energy, protein, fat and carbohydrate according to age group and sex are shown in Table 3. Mean nutrient intake of both age groups of male was low according to RDA (adequate intake - above 90% of RDA, low intake - between 60 & 89% of RDA and very low intake - below 60% of RDA). Mean protein intake of female of both age groups was low, mean energy intake of female of <60 years age group and mean fat intake of 60 years and above age group were low according to RDA. Percent RDA of energy, protein, fat and carbohydrate of women were higher than those of men for the respective age.

Table 3. Mean and % RDA of energy and nutrient intake of type 2 diabetes mellitus patients

	Man (years)		Women (years)	
	20-59	≥60	20-59	≥60
RDA requirement [9]				
for energy (kcal)	2460	2010	1990	1780
for protein (g)	92.3	75.4	74.6	66.8
for fat (g)	68.3	55.9	55.3	49.4
for carbohydrate (g)	369	301.6	298.5	267.1
Mean energy (kcal)	1932.7 ±562.3	1428.3 ±251.1	1593.3 ±386.9	1704.2 ±420.6
% RDA	78.6	71.1	80	95.74
Mean protein (g)	49.0 ±15.1	40.3 ±5.1	47.1 ±33.3	42.9 ±5.9
% RDA	53.20	53.40	63.10	64.20
Mean fat (g)	48.8 ±24.2	33.9 ±7.8	54.7 ±79.9	38.5 ±13.9
% RDA	71.4	60.6	98.9	77.9
Mean carbohydrate(g)	320.03 ±90.9	236.7 ±50.4	281.8 ±228.3	291.3 ±86.9
% RDA	86.7	78.5	94.4	109

Table 4. Percent contribution of protein, fat and carbohydrate to total calories

	Men (years)		Women (years)	
	20-59	≥60	20-59	≥60
Protein	10.2 ±2.1	11.4 ±1.1	10.7 ±2.4	10.4 ±2.1
Fat	22.1 ±7.3	24.2 ±7.2	23.5 ±8.2	20.4 ±5.9
Carbohydrate	66.7 ±6.5	63.4 ±7.5	65.8 ±14.3	67.9 ±6.1

In Table 4, percent contribution of carbohydrate to total calories of subjects with both age groups was around 65%. Percent contribution of protein to total calories was around 10% in all groups and that of fat was around 20-24%.

DISCUSSION

The prevalence of diabetes mellitus is increasing worldwide including developed and developing countries. With the increasing cost of hospitalization, treating complications, loss of productive capacity and the impact on quality of life, nutritional management has been selected as a first step for which nutritional guideline ought

to be established for management in the primary care system. This preliminary study highlighted the situation analysis on dietary habit among the diabetic patients.

Nutrition recommendations for type 2 diabetes mellitus patient are to make reductions in dietary intake of saturated fat, cholesterol, and sodium to reduce glycemia, dyslipidemia and blood pressure. Healthy food choices of them include to choose lean meats, non-fat dairy products, calorie free drinks, liquid oils for cooking instead of solids fats, and non-starchy vegetables and whole grains over processed grain products. High calorie snack foods and desserts like chips, cookies, cakes and full-fat ice cream should be cut back.

In this study, percents of daily consumption of coffee/tea (74.4%), and cold drinks (14.1%) were higher than those of food and beverage consumption of adults living in Pathein (14.9% and 2.3%) and in Mandalay urban area (56.5% and 1.2%), respectively [10]. This indicated that the subjects were found of having sweet foods made with sugar. The simplex carbohydrates, e.g. glucose, sucrose, fructose, with a high glycaemic index exert an additional metabolic challenge to the patients. Therefore, they have to be known to choose calorie free diet drinks instead of regular soda, fruit punch, sweet tea/ coffee and other sugar-sweetened drinks.

The subjects of this study assumed that they should eat special rice, e.g. rice with low quality (*San-kyan*), or rice cooked with discarding surplus water or basmati rice. Diabetic patients should try to choose low and medium glycemic index (GI) foods more often than high GI foods. A glycemic index of 55 or less ranks as low, 56 to 69 as medium, and 70 or more ranks as high. A low glycemic index food will release glucose more slowly and steadily. A high glycemic index food causes a more rapid rise in blood glucose levels and is suitable for energy recovery after endurance exercise or for a person with diabetes experiencing hypoglycemia [11].

The glycemic indexes of rice with low quality are lower than those of rice with high quality, eg. *Paw-sun* rice (so called *San-phyu*). The glycemic index of basmati rice is 72.57 ± 8 but the glycemic index of *Paw-sun* and *Nga-sein* is lower than basmati rice. The GI of *Nga-sein* rice is lowest (glycemic index of *Paw-sun* is 67 ± 3 and *Nga-sein* is 62 ± 2). Basmati rice is well known as rice for the diabetics (*See-cho san*) among population but the glycemic index of this rice is higher than those of most consuming rice in Myanmar like *Paw-sun*, *E-ma-ta* and *Nga-sein* [11, 12, 13]. Six subjects answered that they ate rice cooked with discarding surplus water. However, the caloric content of rice cooked with rice cooker and rice cooked with discarding surplus water does not differ [12].

According to the recommendations of the Malaysian Diabetics Association (1993), the macronutrient contribution to the total calories should be in the ranges of 50-60%, 10-20% and 30% for carbohydrate, protein and fat, respectively. In this study, energy deficit of 527 calories and 582 calories were found in 20-59 years and ≥ 60 years old men and energy deficit of 396 calories and 76 calories were found in 20-59 years and ≥ 60 years old women. Percent contribution of carbohydrate to total calories was $>65\%$. Percent contribution of protein to total calories was 10-12% and that of fat was 20-24% in men and women. Percent contribution of fat to total calories was lower than recommended level in both male and female and that of carbohydrate was higher.

The primary medical nutrition therapy goals for non-insulin dependent diabetes mellitus are to achieve glucose, lipid and blood pressure goals. Hypocaloric diets and weight loss could improve glycemic level and have the potential to improve long-term metabolic control. However, these guidelines need to be depending on blood glucose and lipid levels. The energy requirement should be sufficient to attain and maintain a reasonable body weight for adult.

Conclusion

There were some food taboos among type 2 diabetic patients like eating of *See-cho* rice (Basmati), eating of rice cooked with discarding surplus of water. According to RDA%, distribution of fat to total calories was low but that of protein was sufficient and that of carbohydrate was high. Therefore, type 2 diabetic patients should be educated to eat more vegetables but to reduce carbohydrate like rice and cereal products. In addition, there is a need to correct food taboos. They should be educated to eat low GI foods ($GI \leq 55$) like eating of *Nga-sein* rice in spite of *See-cho* rice and to eat steamed rice to get more vitamin B₁ which is benefit for type 2 diabetes mellitus patients.

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Patients' perspectives on public-private mix initiatives in tuberculosis control

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A considerable number of public-private mix (PPM) initiatives have been launched since the 1990s in Myanmar. This study was conducted to explore the patients' perspectives on PPM initiatives in tuberculosis control. A total of 300 patients, 150 each taking treatment at the National Tuberculosis Program (NTP) clinics and at general practice (GP) clinics, were interviewed from June 2006 to September 2007. A community-based, cross-sectional analytic study was conducted using quantitative and qualitative approaches at five townships in Mandalay Division where PPM initiatives were running concurrently. Relatively older patients were taking treatment at NTP clinics while younger patients at GP clinics, mean ages of 42 ± 16.2 years and 38 ± 15.8 years, respectively ($p=0.020$). Mobile populations were taking treatment at GP clinics while more stable people preferred to take treatment at NTP clinics. Poorer patients were taking treatment at NTP clinics while relatively well-to-do patients at GP clinics ($p=0.018$). Only one-third of studied patients had good knowledge of TB and Directly Observed Treatment Short Course (DOTS) strategy. Patients taking treatment at GP clinics knew better about PPM compared to those at NTP clinics ($p=0.012$). A good program implementation with PPM initiatives and good patients' compliance will ensure effective tuberculosis control.

INTRODUCTION

Myanmar is one of the 22 countries with high burden tuberculosis (TB) and 27 with high multi-drug resistant tuberculosis (MDRTB) in the world. TB is a major public health problem in Myanmar and it is considered as one of the priority diseases in the National Health Plan (2006-2011). To respond to this serious public health problem, the NTP started Public-Private Mix (PPM) piloting linkages with private providers in the late 1990s. A pilot intervention was launched in Shwepyitha Township in the suburbs of Yangon in 1998 [1].

Another PPM initiative called 'Empowerment of General Practitioners (GPs) in TB Control Project' was launched in Kyaukse Township, Mandalay Division, in 2002 [2]. In 2003, PPM-DOTS initiative was started according to the plan for involvement of

private sector in TB control and allowed the involvement of the Myanmar Medical Association (MMA) [3]. Another significant initiative was launched in 2004 by Population Services International (PSI), Myanmar to include DOTS strategy in their existing health franchising scheme, which targeted GPs all over the country [4].

As the results, case detection rates, cure rates and treatment completion rates improved up to World Health Organization (WHO) recommended levels in respective study townships [2, 4]. It is mainly due to vigorous efforts of NTP with involvement of PPM initiatives. However, the sustainability of success after project may need improvement in both service provider side and patients' compliance by understanding and fulfilling perspectives and expectations of TB patients on PPM initiatives. Some studies reported that patient's preference on health care center is associated with quality

of diagnosis and treatment, accessibility, convenient timings [5], preference on health care provider, early diagnosis and appropriate or adequate treatment [6].

Therefore, this study was conducted to explore perspectives of the patients taking anti-TB treatment at public or private sectors on PPM projects in tuberculosis control in selected townships of Upper Myanmar. The specific objectives were to describe the background characteristics of patients taking anti-TB treatment at private and public health facilities, to determine the patients' knowledge and attitudes regarding disease (TB), anti-TB treatment (DOTS), and PPM initiatives, and to explore the patients' expectations from public and private health facilities.

MATERIALS AND METHODS

A cross-sectional analytic study was conducted using quantitative and qualitative approaches at five selected townships, namely Myingyan, Kyaukpadaung, Meikhtela, Kyaukse, and Madayar. The study areas were selected based on: (1) having high TB case load, (2) considerable number of GPs, and (3) presence of PPM initiatives. This study was conducted from June 2006 to September 2007.

Patients taking anti-TB treatment of all categories at selected health facilities during study period were included in the study. There were two types of health facilities, (1) township TB centers (public sector) and (2) GP clinics participating in PPM initiatives of either MMA or PSI (private sector) in the study townships. Thirty patients from each township TB center and two patients from each GP clinic, who were taking anti-TB treatment at the time of survey, were selected by using quota sampling method. A total of 300 patients (150 patients from 5 township TB centers and 150 patients from 75 GP clinics) were interviewed using pre-tested structured questionnaire. The questionnaire included information on background characteristics

of the patients and their knowledge of and attitudes towards TB, DOTS, and PPM initiatives.

To explore patients' expectations from public and private sectors, in-depth interviews (IDIs) were conducted using thematic guidelines. Patients who could answer well in the quantitative survey were purposively selected for IDIs. The IDIs were conducted with 20 TB patients, including 10 patients from township TB centers and 10 patients from GP clinics. Equal numbers of male and female patients were recruited to perform the IDIs.

For quantitative data, Student 't' test for continuous data and chi-square test for categorical data were used to find out the differences in background characteristics, knowledge and attitudes towards TB, DOTS and PPM initiatives between patients taking treatment at public sector and private sector. The critical level of significant was considered at p value <0.05.

For qualitative data management, all IDI sessions were facilitated by principal investigator. A structured interview guide was used to conduct IDI sessions, which focused on the main theme of "patients' expectations from public and private sectors". Sub-themes included patients expectations and opinions on: clinic opening hours, symptomatic drugs, consultation time and number of tablets. All IDI sessions were audio-recorded with consent of participant. The transcripts were written in Myanmar using Win-innwa font in Microsoft Word version 2007. Matrix analysis was done around the main theme and sub-themes. Areas of consensus and divergence were identified in the analysis of this study.

Ethical consideration

The research work was in line with the activities currently practiced by NTP. The ethical approval to conduct this study was obtained from Institutional Ethical Committee of Department of Medical Research (Upper Myanmar).

RESULTS

Background characteristics of TB patients

It was found that the older patients (aged >40 years) were taking treatment at public sector while relatively younger patients (aged around 35 years) were taking treatment at private sector. Association between occupation and choice of treatment facility either public or private sector was also found among TB patients. Mobile populations (e.g. car drivers, and sellers/merchants) were taking treatment at private sector while more stable people (e.g. farmers, government servants) preferred to take treatment at public sector.

Table 1. Background characteristics of TB patients

Background characteristics	TB patients from public sector		TB patients from private sector		p value
	n=150	%	n=150	%	
Age [†] (mean±SD)	41.94±16.19		37.63±15.77		0.020*
<i>Gender</i>					
Male	96	64	103	68.7	0.392
Female	54	36	47	31.3	
<i>Occupation</i>					
Stable occupation	133	88.7	115	76.7	0.006**
Mobile occupation	17	11.3	35	23.3	
<i>Education</i>					
Primary and lower	74	49.3	60	40	0.228
Middle	25	16.7	24	16	
High	24	16.0	37	24.7	
University students and graduated	27	18.0	29	19.3	
Income [†] (mean±SD)	53993±43085		67152±52531		0.018*
Distance [†] (mean±SD)	3.16±3.02		3.29±4.84		0.870
<i>Treatment</i>					
Category I	126	84.0	141	94	0.006**
Category II	24	16.0	9	6	

[†] =Student 't' test, *= $p < 0.05$, **= $p < 0.01$

Patients whose average income was around 50,000 kyats per month preferred more to take treatment at public sector while patients who could earn more were taking treatment at private sector ($p < 0.05$). A higher percentage of Category II TB patients was treated at public sector. However, no gender difference was observed between patients of two sectors. Majority of patients

from both public and private sectors had attained primary education level, 49.3% and 40%, respectively, and it was not statistically significant. Similarly, no significant difference was observed between patients who were taking treatment at private and public sectors regarding the distance from home to health facilities (Table 1).

Patients' knowledge on TB, DOTS, and PPM initiatives

The knowledge on TB eliciting questionnaire included information about signs and symptoms of TB, its cause, mode of transmission, and duration of cough for TB suspect. One score was given for one correct answer. The knowledge level was determined by using the whole-sample mean. Patients who received score less than the whole-sample mean were regarded that they had low knowledge level and those achieved equal to or more than the whole-sample mean were noted that they had high knowledge level. Approximately two-third of the patients from both sectors had low knowledge level on TB and no significant difference was observed in knowledge scores between patients from public sector and private sector.

Knowledge on DOTS was assessed by using a questionnaire which included questions about the duration of treatment for Category I, and Category II TB patients, numbers of sputum recheck, timing for sputum recheck, exact date of next sputum recheck, and completion date for anti-TB treatment. Similar method was adopted to determine the level of knowledge on DOTS. The knowledge levels on DOTS of patients from both sectors were similar ($p > 0.05$). However, regarding awareness on PPM initiatives, the significant higher percentage of patients from private sector (75.3%) had ever heard of PPM initiatives compared with patients from public sector, (62.7%) (Table 2).

One-third of patients taking anti-TB treatment at public sector got information about TB and DOTS from basic health staff

while 42% of patients taking treatment at private sector received information from GPs. The others had known about TB and DOTS from TV, radio or magazines.

Table 2. Patients' knowledge on TB, DOTS, and PPM initiatives

Variable	TB patients from public sector		TB patients from private sector		p value
	n=150	%	n=150	%	
<i>Knowledge on disease (TB)</i>					
High	58	38.7	49	32.7	0.278
Low	92	61.3	101	67.3	
<i>Knowledge on DOTS</i>					
High	51	34.0	65	43.3	0.062
Low	99	66.0	85	56.7	
<i>Awareness of PPM initiative</i>					
Know PPM	94	62.7	113	75.3	0.012*
Don't know PPM	56	37.3	37	24.7	

*=p<0.05

Patients' attitudes towards TB, DOTS, and PPM initiatives

The attitudinal levels of the patients on TB, DOTS and PPM initiatives were divided into two categories, good and poor attitudes. The attitudinal scores for each issue were added and cutoff point was set at whole-sample mean. Patients who received mean and above were regarded as patients with good attitude, while patients below mean were regarded as patients with poor attitude.

Almost all of the patients (91.3% of patients from private sector and 93.3% from public sector) regarded TB as a highly infectious disease. The 88.0% of patients from private sector and 92.6% of patients from public sector strongly disapproved the statement of "TB is an incurable disease". Overall, 87.3% of patients taking treatment at private sector and 80.7% of patients taking treatment at public sector revealed good attitude towards disease (TB). The difference was not statistically significant (Table 3).

Regarding the attitude towards DOTS, 90.6% and 92.6% of patients from private and public sectors were found respectively, regarded DOTS as an excellent strategy for TB control. The 62.7% of patients from

private sector and 62.0% of patients from public sector agreed the statement 'Irregular treatment is dangerous'. There was no significant difference between patients from two sectors concerning attitude towards DOTS.

Table 3. Patients' attitudes on TB, DOTS, and PPM initiatives

Variable	TB patients from public sector		TB patients from private sector		p value
	n=150	%	n=150	%	
<i>Attitudes towards TB</i>					
Good	121	80.7	131	87.3	0.115
Poor	29	19.3	19	12.7	
<i>Attitudes towards DOTS</i>					
Good	98	65.3	100	66.7	0.807
Poor	52	34.7	50	33.3	
<i>Attitudes towards PPM initiative</i>					
Good	77	51.3	148	98.7	0.000**
Poor	73	48.7	2	1.3	

***=p<0.001

A significantly higher proportion of patients with good attitude to PPM initiatives was found among TB patients treated at private sector (p<0.001). The 98.6% of patients treated at private sector regarded GP clinics as a reliable and effective alternative health care provider in TB control. Only 29.3% of the patients taking treatment at public sector regarded GP as an alternative health care provider in TB control.

A remarkable proportion (48.6%) of patients from public sector strongly agreed that 'NTP alone can do TB control without GPs participation'. The main reasons elicited in IDIs were patients in public sector perceived that treatment in public sector is free of charge and public sector is the place for the poor. The overall attitudinal level on PPM initiatives of patients from private sector (98.7%) was significantly higher than that of patients from public sector (51.3%).

Patients' expectations from health facilities

Patients' expectations from public and private health facilities were elicited by conducting IDIs.

Clinic opening hours

Regarding clinic opening hours, majority of patients appreciated the flexible clinic opening hours.

Majority of patients from public sector appreciated the flexible arrangements of public sector. A 28-year-old female primary school teacher taking treatment at township TB center said,

"I'm taking treatment at the hospital (township TB center). Ma Ma (Township Medical Officer) gave me one-month medical leave (from school) when I was found to be sputum positive. I'm also grateful to Sayama (assigned staff at township TB center) for her flexible (time) arrangement for me. I usually go to hospital from school during lunch hours; 12:00 noon-2:00 pm. Sayama is usually at TB center and issued the drugs for me."

However, majority of TB patients from GP clinics said that they need to go to clinic in the clinic opening time, otherwise the GP doctor may be angry with them.

A 40-year-old female patient at GP said,

"Sayar opens the clinic starting from 7:00 am up to 8:00 pm. TB patients need to go to clinic during the clinic opening hours. If we go late, Sayar used to tell like that - You are not emergency patient. We are giving drugs (anti-TB drugs) free of charge. You should come and take drugs in the clinic opening hours!"

Symptomatic drugs

Some patients appreciated to get symptomatic drugs together with anti-TB treatment. A 53-year-old farmer taking anti-TB treatment at township TB center said,

"Cough is very awful for me. In spite of taking drugs (anti-TB treatment), I'm suffering from coughing, especially at night. I complained to Saya (assigned Public Health Supervisor at township TB center). Saya said 'Don't worry. Please continue anti-TB drugs regularly. Those drugs will kill the TB-germs. Cough would subside

automatically. I would like to suggest to give cough remedies for patients like me."

A 43-year-old female seller taking treatment at GP clinic said,

"I felt dizziness, loss of appetite and nausea at the starting days of treatment. Although Saya (GP doctor) gave drugs (anti-TB treatment) for 1 week, I returned to him and complained my sufferings. Saya encouraged me to continue treatment and gave me some injections and tablets. After that, I tolerated on side-effects of treatment. Now, I have been taking anti-TB treatments for 4 months."

Consultation

Some patients expected to get enough consultation time from qualified medical practitioners. A 41-year-old female seller taking treatment at GP clinic said,

"At the start of treatment, doctor gave me a long consultation time --- about half an hour. He explained me about TB, TB-treatment, dangers of irregular treatment etc. Months later, consultation time became shorter --- only about five minutes. He examine me very routinely and continue the drugs. Sometimes, I could not tell my sufferings because of short consultation time."

A 36-year-old male government servant treated at township TB center said,

"Township Medical Officer (TMO) is very busy. He delegates authority to assigned staff. She used to see the TB patients. She is not a medical doctor. I'm afraid of complications (of TB). Once, I suffered from headache. I complained to Sayama that I'm afraid of TB-meningitis. She suggested me to consult TMO. So, I would like to suggest a medical doctor should be assigned at township TB center."

Number of tablets

Patients preferred to take lesser number of tablets without changes throughout the course. A 34-year-old male patient at GP clinic said,

“I'm taking 3 tablets (FDC) daily from GP during first month. During second month, I have to take a total of 14 tablets for one dose. Although doctor said it is the same dose with first 3 tablets, I felt difficulty in swallowing and also suffered from nausea.”

A 28-year-old female patient in township TB center said,

“When my father took anti-TB treatment about 10 years ago, he had to take about 15 tablets daily. He also needed injections. Now, I need to take only 3 tablets a day without injections. It is good for me as I like to take lesser number of tablets.”

DISCUSSION

This study investigated the patients' perspectives on TB, DOTS, and PPM initiatives in order to accelerate and sustain the treatment success rates in TB control. The patients' perspectives involved patients' knowledge of and attitudes towards TB, DOTS, and PPM initiatives as well as patients' expectations from health facilities.

In our study, patients' choice of public or private sector was associated with their background characteristics such as age, occupation, income, and treatment. The older patients were taking treatment at public sector while relatively younger patients were taking at private sector. Mobile populations were taking treatment at private sector while more stable people preferred to take treatment at public sector. Poorer patients preferred more to take treatment at public sector while relatively well-to-do patients were taking treatment at private sector. One local study reported the GPs in Myanmar were utilized by the poorest of the poor in Myanmar [2].

In our study, patients' education level, gender and distance to the nearest health sector were not significant factors for choosing either public or private sector. A study also found that there was no significant difference between males and

females in regards to choice of health facility for anti-TB treatment [7].

In one study, knowledge, attitude and practices on tuberculosis was associated with education, treatment duration, regularity of follow-up and gender of patient [8].

In this study, only one-third of patients (both in public and private sectors) had good knowledge on TB, and DOTS. However, attitudes towards TB and DOTS were good among the patients taking treatment at both public and private sectors. In a report, the majority of participants (80%) had low level knowledge regarding DOTS whereas more than two third of participants had positive attitude towards DOTS [9]. It was also found that only less than half of patients (40%) had knowledge about DOTS [10].

On the other hand, one study which assessed knowledge of TB among patients admitted to medical ward found that more than half of the respondents who were middle adult population (21-60 years of age) had better knowledge on prevention, signs and symptoms of TB and its treatment [11]. Also, most of the participants had high level of knowledge and attitudes towards TB in a study conducted among TB patients at Insein Township Health Centre [12]. These studies reviewed that the attitude toward tuberculosis was better in comparison with a recent study done on patients who were attending and having treatment at NTP, Zone I, Yangon in 1997 [13].

In our study, patients treated at private sector were more aware of PPM initiatives compared to those taking treatment at public sector ($p < 0.05$). The main reasons for choosing public sector were patients in public sector perceived that real free-of-charge treatment could be achieved only in public sector and public sector is the place for the poor. A Study reported that most patients viewed the public sector as a place to obtain free treatment and the private sector as a fee-paying, convenient and better place to seek treatment [14].

Findings from IDIs supported that patients expected flexible and convenient time arrangement from health care providers. Regarding treatment, they expected not only anti-TB drugs but also symptomatic drugs and they preferred lesser number of tablets without changing the number throughout the course. In addition, patients would like to get adequate consultation time from qualified medical doctors. Similar studies, which explored patients' expectations from health facilities, were not found in the available literature.

Based on findings of this study, it is recommended that patients' knowledge levels on causation and mode of transmission of TB, free availability and effectiveness of DOTS, and awareness of PPM initiatives should be improved through Information, Education, and Communication (IEC) services using appropriate mass media channels. Patients' expectations from health care providers ie., convenient clinic opening time, provision of symptomatic drugs, enough consultation time (about thirty minutes) and provision of Fixed Dose Combination (FDC) drugs throughout the course should be fulfilled as much as possible in order to improve the patients' compliance to anti-TB treatment in both public and private sectors.

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Erratum

Establishment and evaluation of in-house one stage prothrombin time test

Myat Mon Oo, Moh Moh Htun, Kyaw Soe, Minn Minn Myint Thu, Kyaw Oo, Tin Tin San, Kyaw Kyaw Aung, Khin Khin Aye & Win Mar

Myanmar Health Sciences Research Journal 2011; 23(1): 51-56.

In this article, there was an error in Table 2. True positive results should have appeared as 19 instead of 20. Therefore, it should have appeared as below:

$$\begin{aligned}\text{Sensitivity} &= \frac{\text{True positive results}}{\text{True positive results} + \text{False negative results}} \times 100 \\ &= \frac{19}{19+1} \times 100\end{aligned}$$

**Prevalence of cervical cytological abnormalities among attendees at
Cervical Cancer Screening Clinic, DMR (Lower Myanmar)**

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The aim of the study was to find out the prevalence of cervical cytological abnormalities in women who attended the Cervical Cancer Screening Clinic, DMR (Lower Myanmar) from July 2008 to July 2010. A total of 1,814 women were screened. Among them, 644 (35.5%) had normal smears, 950 (52.37%) and 116 (6.39%) had inflammatory smears and atypical squamous lesions, respectively. Precancerous lesions such as mild dyskaryosis, koilocytosis, moderate dyskaryosis and severe dyskaryosis were 71 (3.91%), 14 (0.77%), 12 (0.66%) and 1 (0.06%), respectively. Six cases of cervical carcinomas (0.33%) were detected. This study highlighted the prevalence of cervical cytological abnormalities among attendees of the Cervical Cancer Screening Clinic, DMR (Lower Myanmar). Early detection, timely referral, and early treatment could be obtained as the benefit of cervical cytological screening. This study also suggests that National Screening Programmes for cervical cancer should be established in every state and region to cover all women living in the urban and rural areas for early detection of cervical cancer in Myanmar.

INTRODUCTION

Cervical cancer is the second most common form of cancer in women worldwide. More than 80% of these cases are found in developing countries and it is the leading cause of death in these countries [1]. In South East Asia region, 42,538 new cases and approximately 22,594 deaths occurred in 2002 [2]. In Myanmar, cervical cancer constitutes approximately a quarter of new cases of female malignancies every year. The data from Yangon Cancer Registry reports over the past 30 years (1976-2006) showed that out of 56,097 total commonest female cancers, cervical cancer accounts for 13,181 cases. In terms of morbidity and mortality, cancer cervix has a reputation of always being either the commonest or the second commonest female cancers in Myanmar [3].

The prevention of cervical cancer relies on population screening for early detection and

appropriate management of its precursor lesions, cervical intraepithelial neoplasia [4] which if untreated may advance to invasive disease or regress spontaneously [5].

The mortality and morbidity of cervical cancer can be reduced if it is detected in pre-cancerous stage (CIN) by means of cervical cytology screening. Cervical neoplasia (CIN) typically develops over 10 years prior to becoming invasive carcinoma.

While primary prevention would be the ultimate objectives in the control of any cancer, this is not likely to be possible for cervical cancer in the foreseeable future, hence early detection and specifically screening have to be the primary control measures. Even in the developed countries where screening programmes have reduced the incidence and mortality from cervical cancer, continued investment in such programmes is essential to prevent a re-appearance of the disease [6].

The success of cervical cytology screening lies in its relative simplicity, low cost and noninvasive nature. Annual screening reduces the probability of developing invasive carcinoma by over 95%. Most cases of invasive cervical carcinoma occur because there is no screening at an appropriate interval or there is inadequate follow-up for an identified abnormality [7].

The screening is carried out by scraping the exfoliative epithelium of the squamocolumnar junction of the cervix for cytological examination and diagnosis [8].

Effective preventive strategies utilize routine three yearly screening with good follow-up and appropriate management of abnormal smears based on scientifically proven data [9].

Where such programmes are effective, there has been remarkable reduction in both morbidity and mortality from invasive cancer of the cervix, with deaths virtually restricted to those not utilizing these facilities [10].

For the control of cervical cancer, only one proven strategy is cytological screening with appropriate treatment for any abnormalities detected. This study is aimed for early detection of cervical cancer by means of cervical cytology screening.

General objective

- To conduct the cytological screening on cervical cancer in women who attended the Cervical Cancer Screening Clinic, Department of Medical Research (Lower Myanmar).

Specific objectives

- To find out the prevalence of cervical cytological abnormalities in women who attend the Cervical Cancer Screening Clinic, Department of Medical Research (Lower Myanmar)
- To determine the common presenting symptoms of women having cervical cytological abnormalities

MATERIALS AND METHODS

It was a laboratory-based descriptive study. A total of 1,814 women attending the Cervical Cancer Screening Clinic, Department of Medical Research (Lower Myanmar) were screened for cervical cytological abnormalities from July 2008 to July 2010.

The nature of the study was explained thoroughly to the women attending the Cervical Cancer Screening Clinic (DMR-LM). Thorough history taking and physical examination were done and the findings were recorded in the proforma. By using a sterile disposable Cusco's speculum, Pap smears were taken by disposable Ayre's wooden spatula at the transformation zone (TZ) of the cervix without lubrication. Pap smear specimens were immersed immediately in 95% absolute alcohol (ethanol) for 30 minutes and then removed and allowed to air dry. Modified Papanicolaou method i.e., Gill's Haematoxylin V stain, Orange G stain (OG-6) and multiple polychrome stain (EA-50) was used for the staining of Pap smear. After staining, cytopathology diagnosis was done by a pathologist using Bethesda classification.

Interpretation of cervical cytology according to Bethesda System (2001) is as follows:

Negative for intraepithelial lesion or malignancy (NILM)

1. Organisms

- *Trichomonas vaginalis*
- Fungal organisms morphologically consistent with *Candida* species
- Shift in flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with *Actinomyces* species
- Cellular changes consistent with Herpes Simplex virus

2. Other non-neoplastic findings (Optional to report; list not comprehensive)

- Reactive cellular changes associated with

- Inflammation (includes typical repair)
- Radiation
- Intrauterine contraceptive device
- Glandular cells status posthysterectomy
- Atrophy

Epithelial cell abnormalities

1. Squamous cell

- Atypical squamous cells (ASC)
 - of undetermined significance (ASC-US)
 - cannot exclude HSIL (ASC-H)
- Low-grade squamous intraepithelial lesion (LSIL)
 - encompassing: human papillomavirus i.e., koilocytosis/ mild dysplasia/ cervical intraepithelial neoplasia (CIN I)
- High-grade squamous intraepithelial lesion (HSIL)
 - encompassing: moderate and severe dysplasia (CIN II and CIN III) and carcinoma *in situ*
- Squamous cell carcinoma

2. Glandular cell

- Atypical glandular cells (AGC) (specify endocervical, endometrial, or not otherwise specified)
- Atypical glandular cells, favor neoplastic (specify endocervical or not otherwise specified)
- Endocervical adenocarcinoma *in situ* (AIS)
- Adenocarcinoma

RESULTS

In this study, 1,814 women attending the Cervical Cancer Screening Clinic, DMR (Lower Myanmar) were screened for cervical cytology from July 2008 to July 2010. The prevalence of abnormal cervical cytology was 64.5% (1,170 of 1,814 smears). It was further classified into (i) inflammatory (52.37%) (ii) atypical lesions of the cervix (6.39%) which included (ASCUS, ASCH, AGC) (iii) mild

dyskaryosis (3.91%) (iv) koilocytosis (0.77%) (v) moderate dyskaryosis (0.66%) (vi) severe dyskaryosis (0.06%) and (vii) squamous cell carcinoma (0.33%). The details are shown in Table 1.

Table 1. Prevalence of cervical cytological abnormalities in women who attended the Clinic

Cytological diagnosis	Frequency	%
Normal cervical cytology	644	35.5
Abnormal cervical cytology	1170	64.5
Inflammatory (NILM)	950	52.37
Atypical cases	116	6.39
ASCUS	88	4.85
ASCH	16	0.88
AGC	12	0.06
Mild dyskaryosis (LSIL)	71	3.91
Koilocytosis (LSIL)	14	0.77
Moderate dyskaryosis (HSIL)	12	0.66
Severe dyskaryosis (HSIL)	1	0.66
Squamous cell carcinoma	6	0.33

Table 2. Background characteristics of women with cervical cytological abnormalities

Background characteristics	Atypical cases	Precancer cases (LSIL, HSIL)	Cancer (SCC)	Total (%)
<i>Age group (year)</i>				
21- 40	20	22	2	44 (20)
41- 60	88	70	3	161 (73.2)
>60	8	6	1	15 (6.8)
Total	116	98	6	220 (100)
<i>Age of menarche (year)</i>				
12 - 14	63	44	3	110 (50)
15 - 17	45	48	3	96 (43.6)
18 - 20	8	6	0	14 (6.4)
Total	116	98	6	220 (100)
<i>Age of marriage (year)</i>				
15 - 19	12	16	2	30 (13.6)
20 - 24	60	45	3	108 (49.1)
25 - 29	34	30	1	65 (29.6)
30 - 39	10	7	0	17 (7.7)
Total	116	98	6	220 (100)
<i>Marital status</i>				
Married once	112	94	6	212 (96.4)
>once	2	4	0	6 (2.7)
Single	2	0	0	2 (0.9)
Total	116	98	6	220 (100)
<i>Parity</i>				
≥3	66	60	5	131 (59.5)
<3	50	38	1	89 (40.5)
Total	116	98	6	220 (100)

After exclusion of 950 cases of inflammatory smears, that were negative for intraepithelial lesion or malignancy, the socio-medical characteristics of 220 subjects with cervical cytological abnormalities were reviewed. In this study, the commonest age group having cervical cytological abnormalities was the 41-60 years age group

(73.2%). The percentage was higher in subjects who married between 20-24 years (49.1%) and those who had within 3 children (59.5%). Fifty percent of subjects with cytological abnormalities had earlier menarche at 12-14 years, and 96.4% had single marriage (Table 2).

Table 3. Presenting symptoms of cervical cytological abnormalities

Cytology Dx/ Presenting symptoms	Atypical cases (%)	Precancer (%)	Ca cases (%)	Total (%)
<i>Vaginal discharge</i>				
Yes	34(29.3)	40 (40.8)	4 (66.7)	78 (35.5)
No	82(70.7)	58 (59.2)	2 (33.3)	142 (64.5)
Total	116(100)	98 (100)	6 (100)	220 (100)
<i>Abnormal bleeding per vagina</i>				
Yes	10(8.6)	16 (16.3)	2 (33.3)	28 (12.7)
No	106(91.4)	82 (83.7)	4 (66.7)	192 (87.3)
Total	116(100)	98 (100)	6 (100)	220 (100)
<i>Pain in supra pubic area (SPA)</i>				
Yes	28(24.1)	36 (36.7)	4 (66.7)	68 (30.9)
No	88(75.9)	62 (63.3)	2 (33.3)	152 (69.1)
Total	116 (100)	98 (100)	6 (100)	220 (100)

With respect to the symptoms of women having cervical cytological abnormalities, 78 cases (35.5%) presented with vaginal discharge, 28 cases (12.7%) with abnormal bleeding per vagina and 68 (30.9%) had pain in supra pubic area (Table 3).

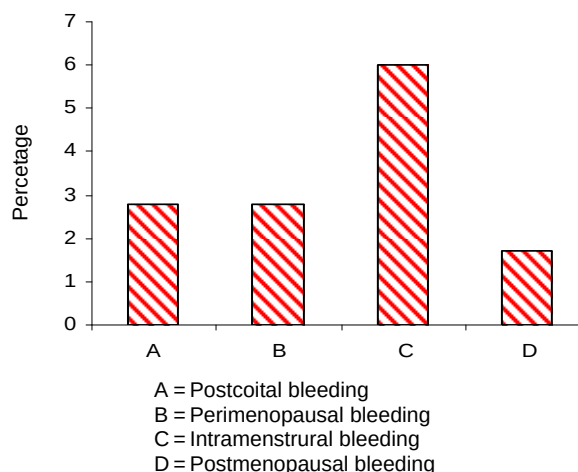


Fig. 1. History of abnormal vaginal bleeding of women having cytological abnormalities

Among 28 (12.7%) women having history of abnormal vaginal bleeding, postcoital bleeding, perimenopausal bleeding, intramenstrual bleeding and postmenopausal bleeding were 6 (2.7%), 6 (2.7%), 13 (5.9%) and 3 (1.4%), respectively (Fig. 1).

DISCUSSION

Cervical screening is estimated to prevent up to 80% of cases of cervical cancer in the United Kingdom [11]. Cervical 'Pap smears' are routinely taken for the purpose of screening for cervical cancer since the clinical disease may be prevented by the detection and appropriate management of its precursor, cervical intraepithelial neoplasia (CIN). Regular screening of sexually active women confers an overall public health benefit in reducing morbidity and mortality from the disease. Studies have shown that women between 35-60 years of age are at high risk of developing cervical cancer [4], and similarly in our study, the most common age group for cervical cytological abnormalities was the 41-60 years age group, constituting 73.2% of women with abnormal cervical smears. However, there is a need to screen women below 35 years of age since the invasive disease is increasingly seen in younger women and CIN occurs predominantly in women under 35 years of age [4].

In England, screening commences at 25 years of age and continues until 64, every 3 years until 50, and every 5 years thereafter. In other developed countries, screening begins at 20 years of age [11]. This study recognized that married women as well as women aged 20 years and above or those who have sexual exposure need to go for Pap test. In a community study, the most common age group having CIN was 36-55 years [12].

In this study, most women at the age group of 41-50 years, age of menarche 12-14 years, age at marriage 20-24 years, parity more than 3 were found to have increased percentage of having cervical cytological abnormalities. A community study revealed that women having CIN i.e., LSIL and HSIL were associated with 36-55 years of age, age of menarche within 12-15 years, age of marriage 15-19 years, and having 3-6 children and our findings were consistent with this community study [12].

The prevalence of abnormal cervical cytology at the Cervical Cancer Screening Clinic, DMR (LM) was 64.50% in this study. In a Nigerian study in 2007, the prevalence of abnormal cervical cytology was 70.3% and out of which, inflammation was the commonest abnormality accounted (52.4%) [13].

In our study, inflammatory changes occurring within the cervical epithelium was 52.37%. This finding is consistent with other studies [12, 13]. Atypical lesions of the cervix including atypical squamous cells of undetermined significance (ASC-US), cannot exclude HSIL (ASC-H) and atypical glandular cells (AGC) were 6.39%. The prevalence of precancerous lesions of the cervix as mild dyskaryosis (LSIL), moderate dyskaryosis (HSIL) and severe dyskaryosis (HSIL) were 3.91%, 0.66% and 0.06%, respectively, although this was mostly due to LSIL, a finding similar to that found in other study [13]. The prevalence of cytological evidence of HPV infection i.e. koilocytosis was 0.77%. It has been observed that HPV infection of the cervix occurs in 99.7% of women with cervical cancer [14], there is hence a need to provide facilities to detect the serotype of the infection virus among these women.

Cervical squamous cell carcinoma cases accounted for six cases (0.33%). These patients require colposcopic examination for further evaluation and biopsy under direct vision; however, despite the high prevalence of cervical intraepithelial neoplasia (CIN) in the population, this service is not available in our screening clinic or at peripheral hospitals. But those patients were referred to Central Women Hospital to get early and appropriate management. Moreover, this study indicates early detection and early treatment as the main benefits of screening. Current knowledge also suggests that combined use of Pap smears with cervicography and human papilloma virus (HPV-DNA) testing improves the sensitivity of detecting cervical intraepithelial neoplasia (CIN II & CIN III) [15].

In Myanmar, cervical cancer is a significant health problem among female cancer patients. One study carried out at the Mandalay General Hospital revealed 0.67% of invasive cancer, 12% of dysplasia and 59.3% of infection [16]. A community study revealed 71.76% had inflammatory smear, mild dyskaryosis 5.29%, moderate dyskaryosis 1.18%, severe dyskaryosis 0.59%, and carcinoma 0.59% [12].

Although many asymptomatic women having cervical cytological abnormalities were found out in this study, only 35.45% of women having cervical cytological abnormalities presented with vaginal discharge, 12.73% with abnormal bleeding per vagina and 30.91% pain in SPA (supra pubic area). A community study revealed that 30.76% of subjects presented with abnormal vaginal bleeding and 69.23% with vaginal discharge [12]. Every woman should be screened for cervical cancer at appropriate intervals as cervical screening is a method of preventing cancer by detecting early abnormalities, which left untreated could lead to cervical cancer.

Conclusion

This study highlighted the prevalence of cervical cytological abnormalities among attendees of the Cervical Cancer Screening Clinic, DMR (Lower Myanmar). Early detection, timely referral, early treatment and regular follow-up could be obtained as the benefit of cervical cytological screening. This study also suggests that National Screening Programmes for cervical cancer in every State and Region to cover all women living in the rural and urban areas are needed for the control of cancer cervix in Myanmar.

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Fermentation of the HBsAg-expressed *Hansenula polymorpha* yeast cells for the production of recombinant hepatitis B vaccine

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Fermentation process is an initial and fundamental step in recombinant hepatitis B vaccine production. In this study, working cell banks (WCB) were produced by cultivation of master cell bank (MCB) containing HBsAg-expressed *Hansenula polymorpha* yeast. Three-step cultivation of WCB (seed 1, 2 and 3) were performed in culture flasks containing 0.7% yeast nitrogen base (YNB) and 2% glucose with incubation conditions of 30°C, 200 rpm for 24 hours. Seed fermentation was continued in 5L seed fermentor. Finally, main fermentation was performed in an industrial 50 liter fermentor (B. Braun Bio-stat U-50) at the condition of 25°C and 200 rpm (changing to 250 rpm after 6 times feeding of methanol) with inner pressure of 1.9 bar and aeration rate of 1.5vvm (70L/min). Methanol feeding was done to induce more antigen expression and cell growth during fermentation process. During the fermentation period, the culture samples were collected twelve-hourly before harvesting the cells for 93.8 hours and were determined for cell mass, cell contamination, HBsAg content and protein concentration. The specific growth rate of the HBsAg-expressed *Hansenula polymorpha* yeast in fermentation process was calculated. It was found that at the end of fermentation time recombinant HBsAg cell mass and protein concentration were 124.5 g/liter and 12.961 mg/ml, respectively, with no contamination. The findings of this study strongly suggested that the thorough and closed monitoring is needed during fermentation process to achieve the optimal fermentative capacity for recombinant HBsAg production.

INTRODUCTION

Fermentation process is an initial and fundamental step in recombinant hepatitis B vaccine production. Recombinant hepatitis B vaccine was produced by using the HBsAg-expressed *Hansenula polymorpha* yeast cells. In this study, the whole fermentation process was started from a single vial of working cell bank. Working cell banks (WCB) were produced by cultivation of master cell bank (MCB), containing HBsAg-expressed *Hansenula polymorpha* yeast. In order to improve the production of hepatitis B virus pre-S2 antigen by the methyltrophic yeast *Hansenula polymorpha*, antigen production was done in two steps: first, three-step cultivation of WCB in

glucose (seed 1, 2 and 3) was performed in culture flasks. Seed fermentation was continued in 5L seed fermentor. Finally, main fermentation was performed in an industrial 50 liter fermentor (B. Braun Bio-stat U-50). Methanol feeding was done to induce more antigen expression and cell growth during fermentation process. The aim of this study was to develop a fermentation process leading to high cell biomass and greatly increased antigen production.

MATERIALS AND METHODS

Material

Master cell bank of hepatitis B surface antigen HBsAg-expressed *Hansenula polymorpha* yeast.

Cell cultivation process

Working cell banks (WCB) were produced from cultivation of master cell bank (MCB) (original cell bank of HBsAg-expressed *Hansenula polymorpha* yeast). Cell cultivation was started from WCB. Cell cultivation steps (seed 1, 2, 3) were performed in culture flasks containing 0.7% yeast nitrogen base (YNB) and 2% glucose media. These flasks were incubated in shaker incubator for 24 hours at 200 rpm, 30°C.

Seed fermentation process

Seed fermentation was performed in 5L seed fermentor under automated control of temperature 25°C, vessel pressure and pO₂ at 200 rpm for 24 hours.

Main fermentation process

Main fermentations were performed in industrial 50-liter B. Braun Bio-stat U-50L fermentor. Foam was controlled by adding the anti-foam agent. The following conditions were maintained throughout the fermentation process.

Conditions of main fermentation

25°C, agitation rate 200 rpm, rpm changed to 250 after 6 times feeding of methanol, inner pressure 1.9 bar, aeration 1.5 vvm (70 L/ min).

The following processes were recorded during the fermentation.

(1) Time of culture (2) Change of culture temperature (3) Change of rpm (4) Change of dissolved oxygen (DO) (5) Methanol feeding time. The dissolved oxygen concentrations, aeration rate, pH in medium, agitation rate, temperature and vessel pressure were monitored *in situ* by probe.

Methanol feeding condition

Methanol (0.6%) of total volume/13 min was fed. First feeding was started at 30 minutes after seed inoculation. When the dissolved oxygen (DO) level reached the highest point, two stopwatches were prepared to record the time and DO level. If

DO level did not increase less than 2.0 for 10 minutes, the methanol feeding was started.

Sample collection

During the fermentation time, the samples were collected twelve-hourly for the following tests:

Cell concentration (cell growth)

The cell growth was determined by using spectrophotometer with OD_{600nm}.

Biomass measurement

Twenty milliliters of sample were centrifuged at 3000 rpm for 15 minutes at 4°C. The supernatant was discarded carefully and the tube was placed upside down for 1 minute on filter paper and then the weights of tube and cells were measured and calculated.

Cell contamination

Cell contamination was checked by high power field of light microscope.

HBsAg purification

The samples were checked for HBsAg protein content by Bradford Assay and HBsAg concentration (*in vitro* test) by AUSZYME (EIA) Abbot ELISA method [1]. When the fermentation process was completed, culture broth was harvested and concentration and diafiltration process were performed. Then, the cell cakes were recovered by centrifugation. The total weight of cell cake was recorded and stored at 4°C for further purification steps. Then, the HBsAg purity was checked by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and confirmed by Western Blot analysis.

RESULTS AND DISCUSSION

Table 1 and Fig. 1 show the summary of fermentation conditions for this study. The harvested cells were disrupted and the supernatants were collected to determine the protein analysis (Bradford) and antigen

value (AUSZYME, first generation antigen standard) was measured.

Table 1. Culture conditions and results

Main initial rpm	200
Rpm increasing after 6 times feeding of methanol	200 → 250
Methanol feeding (ml)	7200
Methanol feeding rate (ml/min)	300/13
Aeration (L/min)	60~70
Temperature (°C)	25
Inner pressure (bar)	1.8~1.9
Culture time (hours)	93.8
Cell cake after harvest	5609 g/53 L

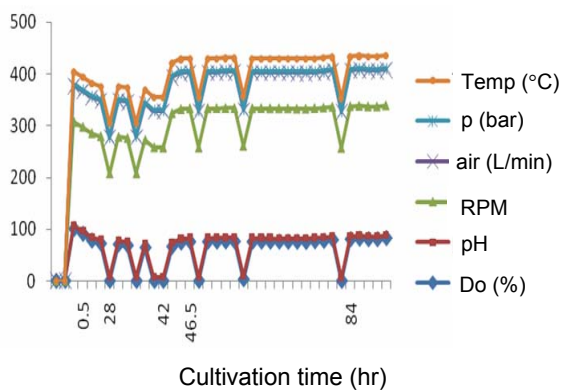


Fig. 1. Fermentation conditions during the cultivation time

Biomass of yeast was increased according to the culture time. With the increase in agitation rate from 200 rpm to 250 rpm, a significant increase in the total protein concentration was observed (Table 2).

Table 2. Changes of specific growth rate, biomass, protein content and HBsAg content at the time of cultivation in fermentation process

Cultivation time (hr)	Bio-mass (g/l)	Protein content (mg/ml)	HBsAg, antigen content (ug/ml)	HBsAg/protein (A)	Specific growth rate (u)	dA/dt	(dA/dt)/u
24	34.2	14.75	0.53	0.04			
36	52	17.18	0.21	0.012	0.03	0.001	0.06
48	72.5	16.48	1.1	0.07	0.03	0.004	0.17
60	87.5	18.62	1.68	0.09	0.016	0.002	0.13
72	100	19.64	3.76	0.19	0.01	0.008	0.76
84	121	14.58	4.86	0.33	0.012	0.012	0.75
94		12.96	5.87	0.45	0.00	0.010	4.19

Specific growth rate (u)=1/X x dX /dt (X=biomass at sampling time)

dA/dt=Changing rate of A according to time

(dA/dt)/u=Changing rate of A according to specific growth rate

The increases of biomass and HBsAg according to the culture time are shown in Fig. 2. Antigen value increased by time, showing that just increase of biomass did not cause decrease of antigen.

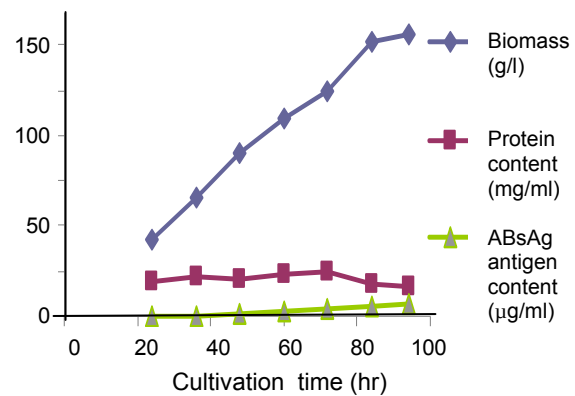


Fig. 2. Changes of biomass (g/lit), HBsAg content and protein content according to the time

Morphological observation of HBsAg-expressed *Hansenula polymorpha* yeast was carried out under optical microscope. It was found that the entire cells showed the same cell morphology proliferation and free from contamination and the dissolved oxygen concentration has been found to influence the productivity of cell. During growth process, notable changes were not observed in this strain and growth curves of all yeast cells showed identical shape.

The antigen values were increased according to the time. HBsAg content was increased and the value of HBsAg was becoming increased at the time of increased rpm. Increasing concentration of protein and HBsAg showed good cultivation of yeast cells, following increasing cell biomass of *Hansenula polymorpha* yeast. Cellular protein production levels decreased during the later stages of the cultivation and were attributed to intracellular protein degradation [2]. Specific growth rate was calculated according to cultivation time. Specific growth rate increased during the cultivation time and decreased at the end of cultivation time.

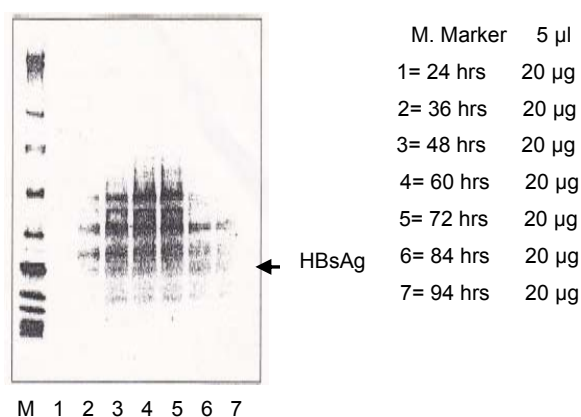


Fig. 3. Identification of HBsAg by SDS-PAGE (8~16%) with silver staining

Fig. 3 shows the presence of HBsAg protein (24kDa) in the cultivating cell samples at various culture times. The final HBsAg products after purification showed the highest yield of antigen content and protein with 100% purity by SDS-PAGE.

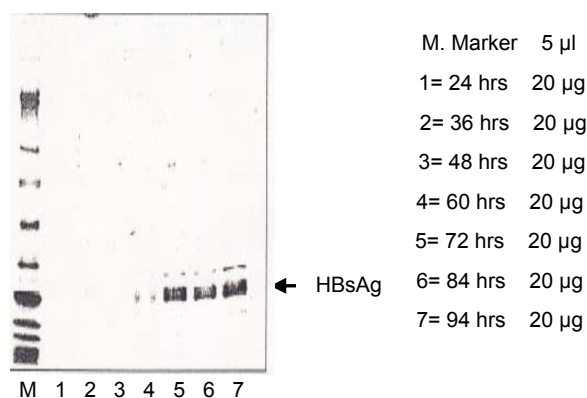


Fig. 4. Confirmation of HBsAg by Western blotting

Immunoblotting indicated that part of the pre-S₂ antigen was present as a 24-KDa degradation product as shown in Fig. 4. In this study, recombinant HBsAg protein and HBsAg content were significantly increased at the end of fermentation process. As antigen value with protein increased by time, it was assumed that just increase of biomass did not cause decrease of antigen.

In this study, increasing speed in the later part of cultivation caused reduction of the interval of adding methanol. Growth rate, specific growth rate and increasing speed of

antigen value are in inverse proportion. Besides, antigen value of final cell is (antigen value is supposed to contain about 1/3 of the amount of protein) about 1/700 of entire amount of protein so if we analyze by protein only then we can know that the growth of antigen is quite low.

Therefore, if the conditions (e.g, enriching methanol starvation, etc.) are applied from the early stage, the antigen value will be high. In order to optimize the growth conditions and more importantly optimize HBsAg production, results in this study indicated that culture at 25°C achieved the highest biomass as well as volumetric HBsAg yields.

These findings are consistent with those of a study which shows that optimum growth temperature, in terms of achieving maximum biomass concentrations and protein production levels for filamentous fungi lies in the range of 28 to 30°C [3]. So, there is a need to find out the optimal fermentation conditions (pH, dissolved oxygen, including nutrient concentration) for cell cultivation in future.

Conclusion

Fermentation is the basic important step to get high concentration of HBsAg-expressed protein (optimal) yields from purification of yeast. The data from this study strongly suggested that the thorough and closed monitoring is needed during fermentation process to achieve the optimal fermentative capacity for recombinant HBsAg production.

Further research should be carried out from basic steps in fermentation to save the manpower, resources and time which should be minimal and within acceptable limits to obtain the optimal HBsAg yields. In terms of highlighting the significant findings of this study, the following points are concluded:

- All inoculation procedures, sampling and sterilizing the transfer lines of the fermentors should be done by the personnel with expertise.

- More attention should be paid to foaming in the main fermentor which can lead to contamination.
- Higher inoculums to obtain maximum cell mass may reduce the lag time in the fermentor.
- Sterilization of seed and main fermentor is an important process before fermentation. Careful attention should be given to these details.
- A short turnaround time of the fermentor can not affect productivity for little or no cost.

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Determination of formalin content in sweetened condensed milk

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The aqueous solution of formaldehyde (formalin) is a useful disinfectant, with lethal effect on most bacteria and fungi. It is also used in industrial processes. Formalin has been reported to be toxic, allergic and carcinogenic to humans. Since formaldehyde is relatively cheap, it has been used in a wide range of processes and products and even wrongly as preservative in some food. Twenty brands of canned sweetened condensed milk and nine uncanned products sold on cost by weight basis were randomly collected, and the formalin content was determined using gas chromatograph equipped with Electron Capture Detector (GC-ECD). The method was established by spiking the sample with 0.1 ppm of formaldehyde, and was found to have a recovery percentage ranging from 81.15% to 105.22%, reproducibility (coefficient of variation) of 3.2%, and the lowest detection limit being 0.052 ppm. Formaldehyde was detected in ten brands (50%) of canned products and eight uncanned products (88.89%). Formalin is generally not permitted to be added to foods directly. However, except for one uncanned product, the contamination levels are lower than the level of naturally occurring formaldehyde for milk, as described by World Health Organization (i.e. 3.3 ppm). The method can be used for evaluating food safety procedures for contamination of formaldehyde in food especially in animal products.

INTRODUCTION

The aqueous solution of formaldehyde (formalin) is a useful disinfectant, with lethal effect on most bacteria and fungi. It is also used in industrial processes including the manufacture of paints, plastic products, paper, textiles, carpets, pesticides and fumigants, particle boards, chipboard and plywood, cosmetics, thermal insulation foams, furniture, biomedical products, leather goods, adhesives, glues and resins. However, formalin has been reported to be toxic, allergic and carcinogenic to human. The toxicity of formaldehyde in methanol's poisoning results from metabolism of formaldehyde by the Cytochrome p 450 enzyme [1, 2].

Formaldehyde is also listed as a suspected gastrointestinal or liver toxicant, immuno toxicant, neurotoxicant, skin or sense organ

toxicant. If it becomes part of food, it turns into formic acid in human body and increases acidity level. More intake of formalin can cause breathing problems, coma and even death. US Environmental Protection Agency and International Agency for Research on Cancer count formalin as one of the cancer-causing agents. At present, formaldehyde is ranked as one of the most hazardous compounds to eco-system and human health. However, formaldehyde is relatively cheap, its use as preservative include a well known Company produced milk formula to prolong shelf life of their perishable products [3, 4].

In Thailand, the Ministry of public Health issues Act No.151 (B.E.2526) specified the contamination of formalin in food is prohibited [5]. Formaldehyde has been banned in cosmetics in both Sweden and Japan. The International Programme on

Chemical Safety (INCHEM) and Centre for Food Safety (Hong Kong) describes that naturally occurring formaldehyde in dairy products is up to 3.3 mg/kg [6, 7]. Therefore, it is important to analyze formalin content in processed food.

In Myanmar, condensed milk is available all as canned products or uncanned, locally produced products sold on cost by weight basics.

General objective

- To determine formalin content in processed food

Specific objectives

- To determine formalin content in sweetened condensed milk with various brand names, produced locally as well as imported.
- To establish a simple and effective method for analyzing formalin content in sweetened condensed milk and evaporated creamer.

MATERIALS AND METHODS

Study design

Laboratory study

Sampling

Twenty-nine brands of sweetened condensed milk included both those imported and those produced locally were collected. Among them, 20 were canned products and the rests were those produced and sold locally by weight.

Reagents and standard

1. n-Hexane-LC grade
2. Formaldehyde - 37% by weight/weight
3. 2,4-Dinitrophenylhydrazine reagent-1.4 g
2, 4-dinitrophenylhydrazine dissolved in 100 ml 2 N hydrochloric acid

Sample preparation and extraction

Sweetened condensed milk sample (2 g) was weighed and placed in a 25 ml beaker. Warm distilled water was added and stirred

to dissolve. And then it was adjusted to be exactly 15 ml.

Two milliliters diluted milk, 4 ml hexane and 2 ml 2, 4-dinitrophenylhydrazine reagent were placed in screw-cap vial, and stirred vigorously with a magnetic stirrer for 30 minutes at room temperature. Emulsified mixture was filtered through thin layer (3-5 mm) of Celite and washed 3 times with successive 4 ml portion of hexane. The organic layer was separated and washed 3 times with successive 5 ml portions of water. The hexane was separated and evaporated to 2 ml. This is the test solution used for injection in the Gas Chromatograph (GC) [8].

Sample analysis using GC

Formalin contents in sweetened condensed milk samples were analyzed by GC using Electron Capture Detector (ECD) [8].

The Gas chromatograph parameters are as follow:

Injector temperature:	250°C
Detector temperature:	280°C
Oven temperature:	Initial temperature 110°C, held for 6 min; then increasing at 15°C/ min to 280°C, held for 1 min
Column:	Optima-5 capillary column; 25mx0.25mm i.d.x 0.25µm film thickness
Carrier gas:	Nitrogen

RESULTS AND DISCUSSION

Twenty canned products and 9 uncanned products were analyzed with GC/ECD. Description of samples together with formalin level detected is shown in Table 1. Among 20 canned products, 10 samples were lower than the lowest detection limit (<0.052 mg/kg), trace amount of formalin was detected in 8 samples (0.241-0.627 mg/kg) and 2 were high level but lower than (3.3 mg/kg) (Table 1), a naturally occurring formaldehyde level described in INCHEM, IPCS, WHO and Centre for Food Safety (Hong Kong) [6, 7].

Table 1. Number of sweetened condensed milk samples together with formalin detected

Type of samples	Samples analyzed (n)	Samples contaminated (n) (%)	Contamination level (mg/kg)	Remark
Samples with can	20	10 (50)	<0.052	Negative
		8 (40)	0.241-0.627	Trace
		2 (10)	2.930-3.251	High
Samples sold by weight (uncanned)	9	1 (11.11)	<0.052	Negative
		7 (77.78)	1.039-3.189	High
		1 (11.11)	4.023	Higher

Among 9 uncanned products sold by weight packets, 1 sample was lower than the lowest detection limit (<0.052 mg/kg) and 1 sample was higher than (3.3 mg/kg) and the rests were 1.039-3.189 mg/kg.

The U.S. Environmental Protection Agency has established a chronic oral reference dose of 0.2 mg/kg bw/day for formaldehyde though it is often difficult to estimate the daily intake of formaldehyde from food. The World Health Organization (WHO) estimated that the daily intake of formaldehyde in food may range between 1.5 and 14 mg in average adult [9]. Other oversea report suggested that if the major contribution is from food, an adult may be exposed up to about 10.55 mg formaldehyde per day.

In the present study, the formalin content in uncanned products was higher than canned products. It seems to indicate that canned sample approved by the FDA to be safer than uncanned products produced by cottage industries. Regarding the method used for formaldehyde analysis in condensed milk the sample spiked with 0.1 mg/kg of formaldehyde had a recovery percentage in the range of 81.15% to 105.22%. The percentage of coefficient variation was 3.2%. The lowest detection limit was 0.052 mg/kg.

The chromatogram of serial concentrations of formaldehyde is shown in Fig.1 and linearity is also satisfactory. It is shown in Fig. 2. This indicates the validity of the present method to be a reliable one for monitoring of formalin content in condensed milk.

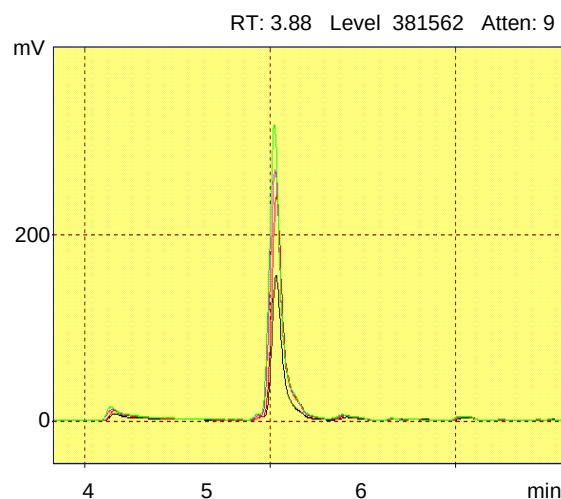


Fig. 1. Chromatogram for different concentrations of formaldehyde

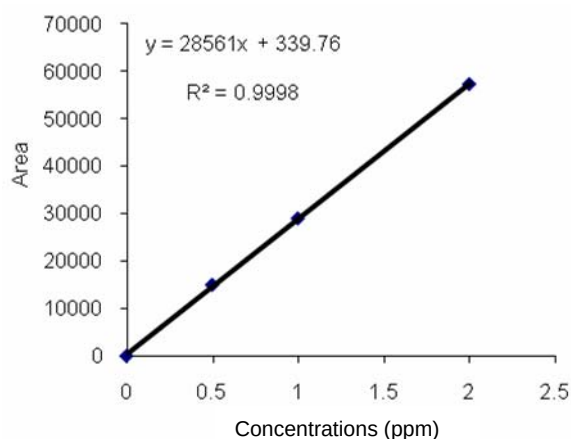


Fig. 2. Standard curve for various concentrations of formalin

Conclusion

Formaldehyde-based solutions are used in embalming dead body to disinfect and temporarily preserve human remains. Its ability to fix tissue is produced firmness of flesh in an embalmed body. Formaldehyde is used as a food preservative illegally since it can prolong the shelf-life of a food by protecting against deterioration caused by microorganisms. Formalin has many negative impacts on human health. If it becomes part of food, it turns into formic acid in human body and increases acidity level [4]. Formalin is not permitted to be added to food directly [10]. Since formalin can affect serious implications on human health it is considered as a carcinogen. The

U.S. Environmental Protection Agency has established a chronic oral reference dose of 0.2 mg/kg b.w/day for formaldehyde though it is often difficult to estimate the daily intake of formaldehyde from food. The World Health Organization (WHO) estimated that the daily intake of formaldehyde in food may range between 1.5 and 14 mg in average adult [9]. Monitoring of food and educating manufacturers and consumers should be carried out to prevent the harmful effect on human health caused by formaldehyde.

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**Knowledge on cervical cancer and opinion towards screening service
among attendees at Cervical Cancer Screening Clinic,
Department of Medical Research (Lower Myanmar)**

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A cross-sectional descriptive study was conducted with the aim of exploring knowledge on cervical cancer and opinion towards screening service among attendees at Cervical Cancer Screening Clinic, Department of Medical Research (Lower Myanmar) from March to September 2010. A total of 151 women aged 21-65 years involved in the study. The mean age was 42 years (SD=9). Most of them were dependent, graduated and married. They got information about this clinic mostly from their friends or relatives (45%). Around 44% of respondents did not know human papilloma virus (HPV) as the major risk factor of cervical cancer. White discharge and abnormal bleeding per vagina were mentioned by 56.3% and 43% of women, respectively, while those who did not know any symptom occupied one fifth of the respondents. Number of sexual partners and number of pregnancies were indicated as main risks of cervical cancer (57%, 23.2%). Almost all of them said that married women should undergo screening and early treatment was stated as the main benefit of screening. Majority (62.3%) got low level of knowledge score, (33.1%) got median and only (4.6%) got high knowledge score. More than half of the respondent indicated TV/video as their main source of knowledge about cervical cancer whereas only 16% mentioned health staff. All showed positive opinion towards screening service they received. As most of the attendees have low level of knowledge score on cervical cancer, clinic based information, education and communication sessions are to be strengthened.

INTRODUCTION

Cervical cancer is the second most common cancer in women worldwide. More than 80% of these cases are found in developing countries and being the leading cause of death in these countries [1]. In South East Asia region, an estimation of 179,500 new cases and round about 101,600 deaths occurred in 2000 [2]. Although cervical cancer is the third leading cause of death in women worldwide, it is a preventable disease [3]. Incidence and mortality of cervical cancer in developed countries have been declining since 1960s due to widespread screening. However, burden of cervical cancer is increasing in developing

countries mainly due to lack of screening [1, 4]. Cervical cancer is the commonest female cancer in Myanmar and its occurrence is the highest among women aged between 15 and 44 years. In Myanmar, 17.92 million women aged 15 years and above are at risk of developing cervical cancer [5]. However, effective cervical screening coverage for women aged 25-64 years was approximately 1% in Myanmar [6].

Success of the screening service depends on providing high quality services that meet women's needs and expectation. Factors reducing women's participation in cervical screening programme include lack of knowledge of cervical cancer and its risk factors, poor awareness of the indications

and benefits of the cervical smear test, fear of pain and embarrassment, anxiety caused by receiving abnormal smear test result and need for additional information [6]. Women will not attend the screening clinic if they think they will be poorly treated. Participation of attendees in screening programme is one important point in sustaining and improving the programme.

In Myanmar, cervical cancer screening service is available at tertiary hospitals and polyclinics. The Cervical Cancer Screening Clinic at DMR (LM) has been established since July 2008. This screening service is provided at no cost and within one and a half year, a total of more than 1,300 women (average - 72 clients per month) aged 20-70 years with various backgrounds were screened. The clinic opens two days per week from 10:00 am to 12:00 noon. The screening service is provided by trained medical officers and laboratory technicians from DMR (LM). A team for one clinic day contains 2 or 3 medical officers and 2 laboratory technicians.

All women who attend this clinic are first asked their socio-demographic background, obstetric and gynaecological history and reason for coming to this clinic (for check-up or due to suffering from some gynaecological symptoms). Then they undergo pap test. Taking clinical history, pap smear, providing smear result and referring those who need to seek further management are done by the medical officers. Smear results are provided two weeks after pap test.

Information about this clinic is publicized in weekly issued local journals. Although the numbers of clients increases within a short period, there is no review of knowledge on cervical cancer and opinion towards screening service of clinic attendees. It is necessary to explore whether the clinic attendees have adequate knowledge or not as they are the likely group with ability to disseminate the correct knowledge to their immediate networks. Moreover, their

adequate knowledge may lead to regular follow-ups and treatment compliance. Furthermore, exploring opinion of the attendees on the service is also important for the improvement of the screening service.

General objective

- To determine the knowledge on cervical cancer and opinion on screening service among attendees of Cervical Cancer Screening Clinic, Department of Medical Research (Lower Myanmar)

Specific objectives

- To assess the knowledge on cervical cancer, its risk factors, indications and benefits of screening among women attending Cervical Cancer Screening Clinic, Department of Medical Research (Lower Myanmar)
- To explore the opinion of clients on the screening service provided by Cervical Cancer Screening Clinic, Department of Medical Research (Lower Myanmar)
- To find out the factors influencing knowledge scores on cervical cancer among clinic attendees

MATERIALS AND METHODS

A cross-sectional descriptive study was conducted among attendees at Cervical Cancer Screening Clinic, Department of Medical Research (Lower Myanmar) from March to September 2010. We assumed that the proportion of the attendees with satisfactory level of knowledge about cervical cancer and satisfied the screening service would be 85% and calculated sample size was 195 at 95% confidence interval and accuracy level of 0.05. All women who attended the screening clinic during study period and who gave consent were selected consecutively. Numbers of women attending the clinic were found to be varied from day to day ranging from 2 to 12 attendees per day but the numbers found to be reduced in rainy days. A total of 151 attendees were interviewed during the study period. Face-to-face interviews were

conducted by using pre-tested semi-structured questionnaire to assess the knowledge on cervical cancer, its risk factors and benefits of screening. Self-administered questionnaire was used to explore the opinion of the attendees on screening service so that they can express their opinion freely without bias.

The interview was performed at a place with privacy. Data cleaning, coding and analysis were done by using Stata 11.1 software. Descriptive statistics were shown as percentages for categorical variables and mean for continuous variables. Difference in knowledge of attendees among different age groups, educational and occupational status were compared using chi-square test. Knowledge of the respondents was considered to be low if they got below mean knowledge score (7, range 0-18) for 4 knowledge items. And total knowledge score was 18. Knowledge level was categorized into low (0-6) and high (7-18). All response categories on the opinion items were 5 point Likert scale, ranging from strongly agree to strongly disagree with an uncertain category. Opinion score was ranging from 17 to 85. All those who got above neutral score (i.e., 51) were considered to have positive opinion towards screening service. The study protocol was approved by the Institutional Ethical Review Committee, Department of Medical Research (Lower Myanmar).

RESULTS

During the study period of six months, a total of 151 women, aged 21 to 65 years participated in the study. The mean age of attendees was 42±9 years. Most of them were married (94%), graduated (53.6%) and dependent (56.3%). Attendees received the information about this clinic mostly from their friends or relatives (48.3%) followed by old patients (17.2%) and print media (16.6%). Commonest reasons of the women to come to this clinic were just for check-up (67.6%) and suffering from white discharge (27.8%) (Table 1).

Table 1. Socio-demographic characteristics of the respondents

Socio-demographic characteristics	Number	%
<i>Age group (years)</i>		
20 - 29	10	6.6
30 - 39	53	35.1
40 - 49	60	39.7
50 - 59	21	13.9
60+	7	4.6
Mean age		42±9
<i>Education</i>		
Primary	6	4
Secondary	11	7.3
High school	45	29.8
University	8	5.3
Graduate	81	53.6
<i>Occupation</i>		
Professional	3	2
Employee	33	21.9
Business	13	8.6
Seller, handicraft	13	8.6
Odd job	4	2.7
Dependent	85	56.3
<i>Marital status</i>		
Married	142	94
Divorced	2	1.3
Widow	7	4.6
<i>Source of information about this clinic</i>		
General practitioners	8	5.3
Old patients	26	17.2
Friends/relatives	73	48.3
Journal/books/magazines	25	16.6
Health staff	24	15.9
TV/video	10	6.6
<i>Reason for coming to this clinic</i>		
White discharge	42	27.8
Abdominal pain	17	11.3
Back pain	11	7.3
Abnormal bleeding	7	4.6
Just for screening	102	67.6
<i>Frequency of visits</i>		
First	29	19.2
Second	109	72.2
Third	8	5.3
Fourth	3	2
Fifth	1	0.7
Sixth	1	0.7

Regarding the level of knowledge of respondent concerning cervical cancer, its risk factors, indication and benefits of screening, most of them (62%) had low knowledge and 38% had high knowledge. As shown in Table 2, majority mentioned white discharge and abnormal bleeding per vagina (56.3% and 45%, respectively). However, dyspareunia and bleeding after

Table 2. Knowledge on cervical cancer

Factors	Number	%
<i>Regarding symptoms</i>		
Symptomless	4	2.7
White discharge	85	56.3
Abnormal bleeding	68	45
Bleeding after coitus	6	4
Dyspareunia	10	6.6
Don't know	31	20.5
<i>Major risk factor</i>		
Human papilloma virus	15	9.9
Don't know	66	43.7
<i>Other risk factors</i>		
Young age at first coitus	26	17.2
Multiple sexual partner	86	57
Multiple full-term pregnancy	35	23.2
Having STI	26	17.2
Smoking	7	4.6
Aging	6	4
Diet with low fruits and vegetables	5	3.3
Long-term use of contraceptive pills	5	3.3
Family history	9	6
Female hygiene	5	3.3
Don't know	24	16
<i>Those who should undergo screening</i>		
18 years and above women	61	40.4
Women who have sexual exposure	37	24.5
Women with symptoms of cervical cancer	15	9.9
Married women	108	71.5

coitus were indicated by only 6.6% and 4% of the respondents. Very few 2.7% expressed that cervical cancer is usually asymptomatic. Furthermore, those who didn't know any symptom occupied one-fifth of the study population (20.5%). Only 9.9% revealed that human papilloma virus (HPV) as the major risk factor of cervical cancer where most of the respondents (43.7%) said they don't know it. But 73.5% can mention at least one risk factor of cervical cancer apart from HPV in which multiple sexual partners was pointed out by more than half of the respondents (57%).

Other risk factors like multiple pregnancies, young age at first coitus, having STI and smoking were indicated by small proportion of the clients (23.2%, 17.2%, 17.2% and 4.6%, respectively). Most of the respondents (71.5%) said that all married women should undergo cervical screening. As for the benefits of screening, early treatment and early

detection of cervical cancer were answered by 85.4% and 32.5% of the attendees.

Table 3 provided the information concerning level of knowledge among different socio-demographic groups concerning cervical cancer, its risk factors, indication and benefits of screening. Among the low knowledge group, majority were between 40-49 years of age (37.2%) followed by 30-39 years (35.1%), graduate (44.7%) and dependent (50%). Regarding the frequency of visit, about 37% of attendees of both first visit and subsequent visits were found to have low knowledge.

Table 3. Factors influencing knowledge scores on cervical cancer

Items	Knowledge level	
	Low (%)	High (%)
<i>Age group (years)</i>		
20 - 29	8 (8.5)	2 (3.5)
30 - 39	33 (35.1)	20 (35)
40 - 49	35 (37.2)	25 (43.9)
50 - 59	14 (14.9)	7 (12.3)
60+	4 (4.3)	3 (5.3)
(Chi square=1.9, df=4, p=0.7)		
<i>Education</i>		
Primary	5 (5.3)	1 (1.8)
Middle	10 (10.6)	1 (1.8)
High school	32 (34)	13 (22.8)
University	5 (5.3)	3 (5.1)
Graduate	42 (44.7)	39 (68.4)
(Chi square=10.2, df=4, p= 0.03)		
<i>Occupation</i>		
Professional	1 (1.1)	2 (3.5)
Employee	26 (27.7)	7 (11.3)
Business	8 (8.5)	5 (8.8)
Seller, handicraft	8 (8.5)	5 (8.8)
Odd job	4 (4.3)	0 (0)
Dependent	47 (50)	38 (66.7)
(Chi square=13.9, df=6, p=0.03)		
<i>Frequency of visit</i>		
First visit (n=29)	11 (37.9)	18 (62.1)
Subsequent visits (n=122)	46 (37.7)	76 (62.3)
(Chi square=0.0005, df=1, p=0.98)		

Considering the source of knowledge of cervical cancer, TV/video was stated by half of the clients followed by print media (45.7%) and friends/relatives (42.4%) whereas health staff was indicated by only (15.9%).

All of the respondents showed positive perception towards the screening service (score: 52-83). But more than one fifth of

Table 4. Opinion of the attendees towards screening service

Items (Multiple response)	Strongly Agree (%)	Agree (%)	Neutral (%)	Disagree (%)	Strongly disagree (%)
Clinic hours	25.6	69.1	0	4	1.3
Clinic days	15	70.4	1.3	10.2	4
Waiting time	25.8	64.2	0.7	8	1.3
Comfort	27	70.7	1.0	1.3	0
Regarding staff	10.5	85.3	0.7	3.2	0.3
Explaining the procedure	13.2	58.9	4	21.9	2
Screening service	30.2	67.4	0.5	1.7	0.2

the attendees (23.9%) said they were not explained about the procedure of taking pap smears. And a few (15%) stated that clinic days were not convenient for them and 10% expressed waiting time at the clinic took longer than they expected. Almost all of the respondents revealed that they wanted to come back to this clinic for follow-up visit (96%) and also want to recommend this clinic to others (98%) (Table 4).

DISCUSSION

All participants in this study were clients of cervical cancer screening clinic. Most of them came to this clinic with the advice of their friends or relatives and old clients. Majority were educated but most of the respondents had low knowledge on cervical cancer and its risk factors. This might be due to the fact that they came to this clinic not because they were aware of the danger of cervical cancer but because this clinic provided screening service at no cost.

This study found out that although the relationship between number of sexual partners and cervical cancer was known, other risk factors like age of first coitus, numbers of pregnancy, smoking, diet, family history and female hygiene were not recognized largely and very little were aware of the link between HPV and cervical cancer. Similar findings were observed from a study done in Ghanaian collage students. The Ghanaian study revealed that only a few percentages could mention HPV,

smoking and family history as the risk factors but nearly half of the study population were aware of the link between sex and cervical cancer [7]. A study in Myanmar found out that more than three-fourth of the respondents considered female hygiene as the major risk factor and more than half of them knew factors related to sex [8].

However, a Belgium study expressed that over 75% of women attending ambulatory gynecology clinic knew HPV cause cervical cancer one year after introduction of HPV vaccine [9]. Having awareness on the relationship between multiple sexual partners and cervical cancer was a positive finding to encourage preventive activities. However, many respondents were not aware of other risk factors. Therefore, health education activities should be emphasized on those risk factors.

Regarding the symptoms, white discharge and abnormal bleeding per vagina were revealed by most of the attendees as these were the significant symptoms that could be easily noticed. Similarly, they were considered as the commonest symptoms in the previous local study [8]. But a few knew other important symptoms like dyspareunia and bleeding after coitus. Only very small proportion could mention asymptomatic and one-fifth of the study population did not know any symptoms. A study conducted among Malaysian women found out that half of the participants knew the symptoms [10]. Therefore, it can be said that respondents from this study had lower level of knowledge on symptoms of cervical cancer compared to Malaysian study.

Respondents recognized that married women as well as women aged 18 years and above or those who have sexual exposure need to go for Pap test. Moreover, they could indicate early treatment and early detection as the main benefits of screening. Many women from Malaysian study believed that Pap test was only for married women and failed to realize early detection of precancerous lesion as the benefit of

screening [10]. Therefore, these were the positive findings to promote screening service.

All of the participants showed positive perception towards screening service. This might be due to the fact that this screening service was provided at no cost. The perception may not be the same if the service is not available free-of-charge. Some said they were not explained about the procedure of taking Pap smears. Although the clinic opens on weekdays, clinic hours and days were found to be convenient for most of the respondents; this was due to the fact that majority of them were dependent. However, a few stated that they had to wait at the clinic more than they expected.

In conclusion, although the attendees of the screening clinic were expected to have higher knowledge on cervical cancer than other women in the community, after reviewing some studies done in the community, it was found out that knowledge level of participants were not much higher than those in community. Therefore, health education session should be established at this screening clinic. Moreover, although all attendees showed positive perception towards the screening service, this study expressed some requirements like prolonged waiting time and explanation about the procedure. Therefore, it is suggested to review the requirements so as to provide better service to the attendees.

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Prevalence of hepatitis B markers in novices and monks residing in a monastery

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Sera samples from 252 novices and monks residing in a monastery in Insein Township in Yangon Division, were screened for the presence of hepatitis B markers. HBsAg (hepatitis B surface antigen), anti-HBs (antibody to hepatitis B surface antigen) and anti-HBc IgG (antibody to hepatitis B core antigen) were determined using immunoassays. Hepatitis B surface antigen was detected in 17.5%, anti-HBs in 32.5% and anti-HBc IgG was detected in 33.7% of the monks and novices. It was found that the prevalence rate of HBsAg was higher than that of general population.

INTRODUCTION

The World Health Organization (WHO) estimates that 2,000 million people (one third of the world's population) have been infected by hepatitis B virus (HBV) worldwide [1]. Approximately 400 million are chronically infected carriers and the annual death rate exceeds a million among the infected individuals [2]. Hepatitis B infection is endemic in developing countries, with an estimated 80 million HBV carriers in the South East Asia Region which is approximately 7% of the regional population [3].

In Myanmar, based on research findings, the prevalence of HBsAg varies between 10-15% [4] and the infection rate is 35% among the general population and 60% among the health care personnel which is included in the high-risk group [5].

Hepatitis B is transmitted through perinatal exposure [6], sexual contact [7], exposure to blood and blood products, organ/tissue transplantation and also by exposure of unknown origin in which the route of transmission cannot be identified [8]. Most adult patients recover completely from their HBV infection, but others, about 5 to 10%, will not clear the virus and will progress to become asymptomatic carriers or develop

chronic hepatitis possibly resulting in cirrhosis and/or liver cancer [9].

This study was undertaken to determine the prevalence of hepatitis B markers among the monks residing in the same monastery. The monks were chosen as the subjects as they can be considered as high-risk group because in addition to living an institutionalized life with other monks, the common practice of shaving the head could be one of the risk factors for transmission of hepatitis B infection.

MATERIALS AND METHODS

Subjects

We carried out a cross-sectional survey on 252 novices and monks residing at a monastery in Insein Township in Yangon Division. The study population included 141 novices (20 years and below) and 111 monks (21 years and above). None of the subjects had been immunized by hepatitis B vaccine previously.

Field study

After obtaining informed consent, three milliliters of blood were collected from each subject under aseptic conditions. Blood samples were centrifuged at 3000 rpm for 10 minutes and sera were separated and

stored at -20°C at the Vaccine Production and Distribution Division of the Department of Medical Research (Lower Myanmar) till further analyses.

Laboratory tests

Sera samples were tested for the presence of hepatitis B surface antigen (HBsAg) using the in-house DMR hepatitis B surface antigen ELISA kit according to the Instruction Manual [10]. Sera samples that were negative for HBsAg were tested for the presence of antibody to hepatitis B surface antigen (anti-HBs) by the DAINA SCREEN AUSAB, an immunochromatographic assay from the Abbott Laboratories, IL, USA [11]. Sera samples that were negative for both hepatitis B markers (HBsAg and anti-HBs) were tested for the presence of IgG antibodies to hepatitis B core antigen (IgG anti-HBc) by Immunocomb for IgG antibodies to hepatitis B core antigen, a solid phase enzyme immunoassay (EIA) from Organics, Israel [12]. Data entry and analysis was carried out using MINI TAB 14 Statistical Package, USA. Univariate analysis was carried out to describe the data and Chi squared tests were carried out to determine the bivariate differences.

RESULTS

Age, sex and study groups

A total of 252 subjects within the age range of 11 to 52 years with the mean age of 20.94±5.66 years participated in the study.

HBsAg prevalence

The overall HBsAg prevalence rate was 17.5% (44 of 252). The HBsAg prevalence was higher among the novices (21.3%) than the monks (12.6%) although the difference was not statistically significant ($p < 1.028$) (Table 1).

Prevalence of anti-HBs

There was no significant difference in the prevalence rate of anti-HBs among the monks (36%) and the novices (29.8%). The overall anti-HBs prevalence was 32.5%.

Prevalence of anti-HBc Ig G

The prevalence of anti-HBc IgG was similar among the novices and monks (34% vs. 33.4%) with an overall prevalence rate of 33.7% (Table 1).

Table 1. Prevalence of hepatitis B markers in novices and monks

Age (yrs)	Subjects (n)	HBsAg (%)	Anti-HBs (%)	Anti-HBc IgG (%)	No markers (%)
<20	141	30 (21.3)	42 (29.8)	48 (34.0)	21(14.9)
>20	111	14 (12.6)	40 (36.0)	37 (33.4)	20(18.0)
Total	252	44(17.5)	82 (32.5)	85 (33.7)	41(16.3)

DISCUSSION

In this study, sera samples from the monks and novices residing in a monastery in Insein Township, a peri-urban area in Yangon, were tested for the presence of hepatitis B markers (HBsAg, anti-HBs and anti-HBc IgG). The overall prevalence of hepatitis B surface antigen (HBsAg) among the novices and monks was 17.5% and was higher than the HBsAg prevalence rate of 12.5% among the health care personnel [5] and 10-12% among the general population [13]. The age-specific HBsAg prevalence was higher among the novices under the age of 20 years (21.3%) compared to the monks (12.6%) although the difference was not statistically significant.

Although HBsAg is the prototype marker of infection, HBsAg positivity does not indicate replication of virus or chronic hepatitis. The failure to clear HBsAg within six months defines a chronic HBsAg carrier state [14]. However, HBsAg is present in both acute and chronic hepatitis, and acute infection could be confirmed by the presence of high titers of IgM anti-HBc [9]. Since we were unable to test for IgM anti-HBc in this study, we could not confirm whether the novices were suffering from acute infection or whether they were carriers. HBcAg is an intracellular antigen expressed in infected hepatocytes and is not detectable in the serum. Antibody to HBcAg

can be detected throughout the course of the HBV infection and IgM anti-HBc is the only marker of HBV infection during the window period [15]. Although IgM anti-HBc antibodies typically decline to undetectable levels within 6 months, the IgG class (IgG anti-HBc) persists indefinitely as a marker of past HBV infection [16]. Past HBV infection is diagnosed by detection of anti-HBs and anti-HBc IgG. In our study, the prevalence of anti-HBc IgG was similar among the two groups (34% among the novices and 33.4% among the monks), with the overall prevalence of 33.7% which could also be interpreted as past infection.

Anti-HBs is a protective antibody, and the last to appear during HBV infection. It is also a long-lasting antibody and is associated with immunity. The presence of anti-HBs and anti-HBc (IgG) indicates recovery and immunity in a previously infected individual, whereas a successful vaccination response produces antibody only to HBsAg. The appearance of anti-HBs indicates recovery from infection and protection against reinfection [17]. The overall anti-HBs prevalence in the present study was 32.5% which was higher than the prevalence of anti-HBs among the health care personnel (24%) [5] which is regarded as a high-risk group. The prevalence of anti-HBs was higher among the monks (36.0%) compared to 29.8% among the novices, although the difference was not significant. Findings from the present study indicated that the rate of sero-conversion to anti-HBs was higher among the monks.

In the present study, the overall prevalence for the three hepatitis B markers was 17.5% for HBsAg, 32.5% for anti-HBs and 33.7 % for anti-HBc IgG, respectively. An overall 16.3% (14.9% of the novices and 18% of the monks) had no serological markers for hepatitis B. These subjects are susceptible for acquiring hepatitis B infection and should be immunized.

Findings from an international study showed that the high prevalence of markers

of hepatitis B virus infection among children living in residential institutions indicates that HBV infections have the propensity for spread in an institutional setting. The prevalence of HBV increases with the duration of time spent at the institution and hepatitis B immunization is highly recommended for susceptible residents and the staff [18]. The high prevalence of hepatitis B markers among the monks might be due to living an institutionalized existence in close contact with infected persons and the iatrogenic risk obtained through the common sharing of razor (*thin-done-dah*) used for shaving the head periodically, that can give rise to minor cuts during the process of shaving and could be important factors in transmitting hepatitis B infection from an infected monk to a susceptible novice.

Conclusion

To prevent the transmission of hepatitis B infection among the monks and novices, the common practice of using a common razor (*thin-done-dah*) for shaving heads should be replaced by the use of disposable razors. Health educational talks on hepatitis B infection should be given to the monks and the practice of using disposable razors should be promoted in all monasteries in the country. Susceptible individuals should be recommended for immunization with hepatitis B vaccine.

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Prevalence and correlation of obesity, hypertension and type 2 diabetes mellitus in selected townships of Upper Myanmar

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This community-based, cross-sectional study aimed to find out the prevalence and correlation of common non-communicable diseases (NCDs) in Kyaukse Township (plain area) and Lashio Township (hilly area) from January to August 2009. Out of 3,200 respondents, the prevalence of obesity, hypertension and diabetes mellitus were 77, 215 and 96 per 1,000 of ≥ 18 years old adult population, respectively. The prevalence of obesity, hypertension and diabetes mellitus was 97, 190 and 111 per 1,000 adult population in plain area and 59, 240 and 81 per 1,000 adult population in the hilly area, respectively ($p < 0.05$). The prevalence of hypertension and diabetes mellitus in urban area was 234 and 107 per 1,000 adult population and it was 196 and 85 per 1,000 adult population in rural area, respectively ($p < 0.05$). The obese people were approximately two times more likely to develop hypertension than those with normal body weight (Crude OR=2.0; 95% CI 1.51 to 2.68; $p < 0.005$). The odds of being a diabetic was 3.6 times higher among obese compared to people with normal body weight (Crude OR=3.2; 95% CI 2.28 to 4.46; $p < 0.005$). A significant association between hypertension and diabetes mellitus was also found in this study (Crude OR=1.5; 95% CI 1.17 to 2.01; $p = 0.001$). In conclusion, the common NCDs in Myanmar including obesity, hypertension and type 2 diabetes mellitus were correlated to each other.

INTRODUCTION

The developing countries including Myanmar are confronted with a double burden of diseases, posing as two challenges to resources reserved for health care delivery. The first challenge is the ongoing burden of infectious diseases such as malaria, tuberculosis and HIV/AIDS. The second and new challenge is that of NCDs [1]. Among NCDs, obesity, hypertension and diabetes are common diseases. Those may exist for a long time in the individual without symptoms and may manifest only after causing serious irreversible pathology and complications; may call them 'silent killers'. Emerging burden of NCDs is previously and still mostly neglected in most developing countries. Their prevalence is also increasing in nations of the developing world [1]. Therefore, prevention,

early detection, adequate long-term treatment and control of those conditions should receive high priority. Although the risk factors for NCDs and relationships between those chronic conditions are well recognized, there is a limited understanding of the scope of these problems in Myanmar. Reliable information on common NCDs in Myanmar is essential to contribute towards national health policies for prevention and control of those conditions. In this study, we reported the prevalence and correlation of common NCDs namely obesity, hypertension and type 2 diabetes mellitus in different population groups in Myanmar.

MATERIALS AND METHODS

A community-based, cross-sectional study was conducted from January to August 2009. Multistage sampling method was used.

In the first stage, Kyaukse Township for Mandalay Division and Lashio Township for Northern Shan State were selected as the study areas. At the second stage, wards and villages were selected. For urban areas, updated list of quarters was used as sampling frame. Identification numbers were given to each quarter. Two quarters were selected randomly in each township. For rural areas, two villages where Rural Health Center is located were chosen based on the feasibility of communication. At the third stage, random starting point was chosen on the area map. Next, data collectors went to the chosen point on land and chose the direction to forward by rotating a bottle on the ground. The data collectors went to the street of direction, and collected the required number of consecutive household. Only one respondent in each household was selected from the list of household members by simple random sampling.

The sample size was calculated using the formula: $(n) = z^2 pq/d^2$, valid for cross-sectional prevalence studies. The input criteria for sample size estimation were: $z=1.96$ for (95% confidence interval), p =estimated prevalence of hypertension in Myanmar (0.2) [2], $q=(1-p)=0.8$, $d=0.02$ (margin of error), and design effect was set at 2 (multistage sampling), and the required smallest sample size was 3,074. A total of 3,200 respondents, 1,600 in Lashio Township (800 in urban and 800 in rural) and 1,600 in Kyaukse Township (800 in urban and 800 in rural) involved in the study. The non-response rate was about 8%. The main reason was not available at the time of survey and it was replaced with another household member in the same household.

Face-to-face interviews were conducted using pre-tested questionnaire to assess background characteristics of respondents, life style behaviors, awareness of diseases, and treatment seeking patterns. Informal group discussions were also done with village elders and local informants to assess

the awareness of NCDs in the community. Standardized sphygmomanometers, stethoscopes, measuring tapes, bathroom scales, Uripath urine sugar dipsticks, and Ascensia Elite XL digital glucometers were used for measuring the blood pressure, height, weight, urine sugar and blood glucose levels, respectively. Measurements were carried out by trained research assistants of Department of Medical Research (Upper Myanmar) who have been trained on data collection tools for three days.

Body weight was measured in kilograms (kg) to the nearest one decimal point by using bathroom scale, wearing light cloths without footwear. Height was measured in meters (m) up to the nearest one decimal point by using measuring tape as the respondent rested erect against the vertical wall. Body mass index (BMI) was calculated as body weight (in kilograms) divided by height in square meters ($BMI = kg/m^2$). Obesity was defined as $BMI \geq 30$ [3].

Blood pressure was measured on right arm at sitting position, 5 minutes rest after the interview. The sphygmomanometers were standardized with the standard sphygmomanometer of Epidemiology Research Division of Department of Medical Research (Upper Myanmar). Systolic pressure was recorded at the appearance of first Korotkoff sound and diastolic pressure was recorded at the disappearance of fifth Korotkoff sound. Blood pressure measurement was done at least for 2 times with 5 minutes apart. If there was a difference of 10 mmHg or more, third measurement was done and the result of the first measurement was discarded. Systolic blood pressure of 140 mmHg and above and diastolic blood pressure of 90 mmHg and above regardless of ages of respondents was regarded as hypertensive [4]. Screening for diabetes was conducted by urine sugar test. Urine samples from all respondents were tested for urine sugar with Uripath dipstick. Urine sugar positive cases were confirmed by Fasting Blood Sugar (FBS) test. Diabetic mellitus was defined as $FBS \geq 126$ mg/dl [3].

Ethical consideration

Ethical approval was obtained from Institutional Ethical Committee of Department of Medical Research (Upper Myanmar). Written informed consent was obtained from each and every respondent. Regarding the rescue treatment, all patients suffering from any NCD under study were informed, explained the nature of disease and initial treatment was given. All patients were referred to respective Township Health Departments for further necessary investigations and management.

RESULTS

Background characteristics of respondents

In Kyaukse Township, most of the respondents were Bamar (96.6%) and other races included Indian (2.0%), Chinese (0.6%), Shan (0.5%) and other minor ethnics (0.3%). In Lashio Township, majority were Shan (40.4%) followed by Bamar (23.5%), Kokant (17.3%), Gorakha (Nepalese) (7.9%), Chinese (3.9%), Palaung (3.3%), Indian (2.6%) and other minor ethnic groups (1.1%). The mean age of all respondents was 38.6 ± 14.1 years which ranged from 18 to 89 years. The age distribution of respondents in plain and hilly areas as well as urban and rural areas was similar in this study ($p > 0.05$). Female preponderance was found among respondents (2054 females vs. 1146 males). Most of the respondents (92.9%) were married and the proportion of married respondents was higher in rural compared to urban, 94.1% vs. 91.8%, respectively ($p = 0.009$).

The education levels of respondents residing at plain and urban areas were higher than those residing at hilly and rural areas ($0 < 0.05$). Majority of respondents were farmers (41.2%), followed by laborers (36.0%), dependents/ housewives (14.7%) and government servants (8.1%). There was no significant difference in occupation of respondents in hilly and plain areas ($p = 0.133$).

Personal habits

At the time of study, 15.6% of respondents smoked at least three cigarettes per day and 9.3% drank alcohol daily. Approximately half (54.6%) of the respondents had sweetened beverages and approximately one-third (34.9%) ate salted-fish /salted fish paste (*Nga-pi*) and salted fish sauce (*Ngan-pyar-yae*) every day. Majority of respondents (80.4%) never played sports or any physical exercise.

Prevalence of NCDs

The overall prevalence of obesity, hypertension and diabetes mellitus were 77, 215 and 96 per 1,000 ≥ 18 years adult population, respectively. Differences in prevalence of NCDs in plain versus hilly and urban versus rural areas are shown in Table 1.

Table 1. Prevalence of NCDs in plain versus hilly and urban versus rural areas

NCDs	Plain (n=1600)			Hilly (n=1600)			Overall n=3200	P
	Rural	Urban	All	Rural	Urban	All		
	n=800	800		n=800	800			
Obesity	82	111	97	74	44	59	77	0.000
HT	119	261	190	208	273	240	215	0.001
DM	80	141	111	74	89	81	96	0.005

NCDs=Non-communicable diseases

HT=Hypertension

DM=Diabetes mellitus

The prevalence of obesity, hypertension and diabetes mellitus was 97, 190 and 111 per 1,000 adult population in plain area and it was 59, 240 and 81 per 1,000 adult population in hilly area, respectively ($p < 0.05$). The prevalence of obesity and diabetes mellitus was higher in plain area and the prevalence of hypertension was higher in hilly area ($p < 0.05$).

The prevalence of NCDs was higher in females than in males ($p < 0.05$). The prevalence of obesity, hypertension and diabetes mellitus in females were 83, 261 and 115 per 1,000 adult population, respectively, and 68, 133 and 62 per 1,000 adult population in males. The age-specific prevalence of hypertension was 50 per

1,000 adult population in ≤ 25 years, 183 in 26 to 40 years and 334 in >40 years. The monotonic increment in the prevalence of diabetes mellitus were found with age: 50, 88 and 128 per 1,000 adults in ≤ 25 years, 26 to 40 years and >40 years age groups, respectively ($p < 0.001$). The prevalence of hypertension and diabetes mellitus were increasing with age ($p < 0.001$), however, it was not true for obesity.

Association of NCDs

The prevalence of hypertension among obese were higher than among people with normal body weight, 341 vs. 204 per 1,000 population. The obese people were approximately two times more likely to develop hypertension than people with normal body weight (OR=2.0; 95% CI 1.51 to 2.68; $p < 0.005$). The odds of being a diabetic was significantly higher among obese (OR=3.2; 95% CI 2.28 to 4.46; $p < 0.005$). The obese people were 3.2 times more likely to be hypertensive compared to people with normal body weight.

Moreover, a significant association between hypertension and diabetes mellitus was found in this study (OR=1.5; 95% CI 1.17 to 2.01; $p = 0.001$).

Risk factors for NCDs

Risk factors for obesity

The significant factors associated with an increased likelihood of being obese were staying at plain area compared to those in hilly area (9.7% vs. 5.9%), being other ethnic groups compared to Bamar (9.1% vs. 6.9%), high income compared to those with ≤ 40000 kyats (9.4% vs. 6.2%), family history compared to those without history (17.1% vs. 6.9%), and physical inactivity compared to those taking regular exercise (8.8% vs. 3.7%). (Table 2).

Risk factors for hypertension

The odds of increase in blood pressure was higher among the respondents who live in hilly area compared to those in plain area

Table 2. Significant risk factors for obesity

Factors	Obesity		Crude odds ratio (95%CI)	Adjusted odds ratio (95%CI)
	No (%)	Yes (%)		
Geography				
Hilly	1506 (94.1)	94 (5.9)	1	1
Plain	1445 (90.3)	155 (9.7)	1.7 (1.31 to 2.27)	1.6 (1.19 to 2.05)
Ethnicity				
Bamar	1778 (93.1)	132 (6.9)	1	
Others	1173 (90.9)	117 (9.1)	1.3 (1.02 to1.75)	NS
Family income per month (kyats)				
≤40000	1488 (93.8)	98 (6.2)	1	1
>40000	1463 (90.6)	151 (9.4)	1.6 (1.19 to 2.06)	1.4 (1.08 to 1.84)
Family history				
Yes	238 (82.9)	49 (17.1)	2.8 (1.94 to 3.95)	2.9 (2.02 to 4.02)
No	2713 (93.1)	200 (6.9)	1	1
Exercise				
Yes	604 (96.3)	23 (3.7)	1	1
No	2347 (91.2)	226 (8.8)	2.5 (1.61 to 14.16)	2.3 (1.49 to 3.57)

NS=Not significant

(24% vs. 19%), who live in urban compared to rural area (23.4% vs. 19.6%), being female compared to male (26.1% vs. 13.3%), age >35 years compared to ≤ 35 years age (33.4% vs. 10.0%), low education compared to higher education level ($>$ middle school) (22.5% vs. 19.4%), being dependent compared to worker (27.4% vs. 19.7%), being married compared to single (22.3% vs. 11.1%), grand multi-parity compared to women with <4 children (30.4% vs. 22.2%), high income compared to those with ≤ 40000 kyats (23.6% vs. 18.9%), family history compared to those without history (24.4% vs. 20.4%), preference on salty diet compared to those without preference (26.1% vs. 19.0%), smoker compared to non-smokers (56.0% vs. 15.1%) and physical inactivity compared to those taking exercise (22.7% vs. 16.4%) (Table 3).

Risk factors for diabetes mellitus

The odds of being diabetic patient was higher among respondents who live in plain area compared to those in hilly area

Table 3. Significant risk factors for hypertension

Factors	Hypertension		Crude odds ratio (95%CI)	Adjusted odds ratio (95%CI)
	No (%)	Yes (%)		
Geography				
Plain	1296 (81.0)	304 (19.0)	1	1
Hilly	1216 (76.0)	384 (24.0)	1.4 (1.1 to1.6)	2 (1.4 to 2.8)
Rural and urban				
Rural	1287 (80.4)	313 (19.6)	1	1
Urban	1225 (76.6)	375 (23.4)	1.3 (1.06 to 1.5)	1.6 (1.14 to 2.18)
Gender				
Male	995 (86.7)	153 (13.3)	1	NS
Female	1517 (73.9)	535 (26.1)	2.2 (1.87 to 2.81)	
Age (years)				
≤35	1465 (90.0)	163 (10.0)	1	1
>35	1047 (66.6)	525 (33.4)	4.5 (3.7 to 5.5)	4.8 (3.71 to 6.15)
Education				
>Middle	862 (80.6)	208 (19.4)	1	NS
≤Middle	1650 (77.5)	480 (22.5)	1.2 (1.0 to 1.45)	
Occupation				
Worker	1983 (80.3)	488 (19.7)	1	NS
Dependent	529 (72.6)	200 (27.4)	1.5 (1.26 to 1.86)	
Marital status				
Single	201 (88.9)	25 (11.1)	1	NS
Married	2311 (77.7)	663 (22.3)	2.3 (1.5 to 3.68)	
Parity				
<4	825 (77.8)	235 (22.2)	1	NS
≥4	692 (69.6)	302 (30.4)	1.5 (1.25 to 1.88)	
Family income per month (kyats)				
≤40000	1170 (81.1)	273 (18.9)	1	NS
>40000	1342 (76.4)	415 (23.6)	1.3 (1.11 to 1.58)	
Family history				
Yes	666 (75.6)	215 (24.4)	1.3 (1.04 to 1.52)	1.5 (1.15 to 1.89)
No	1846 (79.6)	473 (20.4)	1	1
Preference on salty diet				
Yes	825 (73.9)	292 (26.1)	1.5 (1.26 to 1.79)	NS
No	1687 (81.0)	396 (19.0)	1	
Smoking				
Yes	220 (44.0)	280 (56.0)	7.1 (5.79 to 8.83)	8.4 (6.15 to11.43)
No	2292 (84.9)	408 (15.1)	1	1
Exercise				
Yes	524 (83.6)	103 (16.4)	1	1
No	1988 (77.3)	585 (22.7)	1.4 (1.2 to 1.8)	2.3 (1.7 to 3.3)

NS= Not significant

(11.1% vs. 8.1%), urban residents compared to rural residents (10.8% vs. 8.4%), being other ethnic groups compared to Bamar (11.1% vs. 8.6%), being female compared to male (11.5% vs. 6.2%), age >35 years compared to ≤35 years age (12.2% vs. 7.1%), being married compared to single (10.0% vs. 4.4%), grand multi-parity compared to women with <4 children (16.0% vs. 7.3%), high income compared to those with ≤40000 kyats (10.7% vs. 8.5%), family history compared to those without history (23.0% vs. 8.4%), preference on sweet food compared to those without preference (11.2% vs. 7.6%), smoker compared to non-smoker (20.0% vs. 7.7%) and lack of exercise compared to those taking exercise (10.1% vs. 7.3%) (Table 4).

Multiple logistic regression analysis

Stepwise multiple logistic regression analysis was done in R-software. The variables significant at 0.05 level in bi-variate analysis were included in basic model. Stepwise selection of models was executed in R-software and the model with lowest Akaike Information Criterion value was finally chosen to describe the combined effect of variables on disease development. The risk of obesity was increased in the respondents who live in plain area, high family income, family history of obesity and physical inactivity.

The factors significantly associated with increased likelihood of being hypertensive were residing in hilly area, urban area, age >35 years, family history, smoking and physical inactivity. The odds of being diabetes mellitus were higher among respondents in urban area, age >35 years, grand multi-parity, family history, smoking and physical inactivity.

Awareness of NCDs in the community

During informal group discussions with local people, majority of participants responded dizziness and sudden fainting attack as symptoms of hypertension and finding ants on urine, and delayed wound healing as symptoms of diabetes. Regarding

Table 4. Significant risk factors for diabetes mellitus

Factors	Diabetes		Crude odds ratio (95%CI)	Adjusted odds ratio (95%CI)
	No (%)	Yes (%)		
Geography				
Hilly	1470 (91.9)	130 (8.1)	1	NS
Plain	1423 (88.9)	177 (11.1)	1.4 (1.1 to 1.79)	
Rural and urban				
Rural	1465 (91.6)	135 (8.4)	1	1
Urban	1428 (89.2)	172 (10.8)	1.3 (1.02 to 1.67)	1.7 (1.24 to 2.19)
Ethnicity				
Bamar	1746 (91.4)	164 (8.6)	1	NS
Others	1147 (88.9)	143 (11.1)	1.3 (1.04 to 1.69)	
Gender				
Male	1077 (93.8)	71 (6.2)	1	NS
Female	1816 (88.5)	236 (11.5)	1.9 (1.49 to 2.64)	
Age (years)				
≤35	1513 (92.9)	115 (7.1)	1	1
>35	1380 (87.8)	192 (12.2)	1.8 (1.43 to 2.35)	1.4 (1.07 to 1.92)
Marital status				
Single	216 (95.6)	10 (4.4)	1	NS
Marred	2677 (90.0)	297 (10.0)	2.4 (1.26 to 5.13)	
Parity				
<4	983 (92.7)	77 (7.3)	1	1
≥4	835 (84.0)	159 (16.0)	2.4 (1.81 to 3.28)	2.2 (1.63 to 2.97)
Family income per month (kyats)				
≤40000	1451 (91.5)	135 (8.5)	1	NS
>40000	1442 (89.3)	172 (10.7)	1.3 (1.0 to 1.64)	
Family history				
Yes	207 (77.0)	62 (23.0)	3.3 (2.36 to 4.52)	3.2 (2.14 to 4.7)
No	2686 (91.6)	245 (8.4)	1	1
Preference on sweet food				
Yes	1552 (88.8)	196 (11.2)	1.5 (1.19 to 1.97)	NS
No	1341 (92.4)	111 (7.6)	1	
Smoking				
Yes	400 (80.0)	100 (20.0)	3.0 (2.29 to 3.93)	2.7 (1.9 to 3.68)
No	2493 (92.3)	207 (7.7)	1	1
Exercise				
Yes	581 (92.7)	46 (7.3)	1	1
No	2312 (89.9)	261 (10.1)	1.4 (1.1 to 2.0)	1.6 (1.1 to 2.5)

NS= Not significant

the risk factors for NCDs, participants reported that family history, sedentary lifestyle and overeating were responsible for obesity, preference to fatty diet, salty diet and aggressive behavior for hypertension, and overeating sweet and family history for diabetes. Knowledge on complications of NCDs was also investigated and participants reported affect on beauty especially in women, blockage in the blood vessels with fats and knee joint pain as the complications of obesity, sudden rupture of brain vessels and inability to move some parts of body as the complications of hypertension, and loss of function of kidneys, numbness of extremities and impotency as the complications of diabetes.

DISCUSSION

In this study, the overall prevalence of obesity, hypertension and diabetes mellitus were 77, 215 and 96 per 1,000 in ≥18years old adult population, respectively. The prevalence of hypertension varied from 4.5% to 31% in India, 0.6% to 19.4% in Indonesia and 5% to 20% in Nepal. The prevalence of obesity was between 5.5% to 16.6% in India and 15% in Sri Lanka. The prevalence of diabetes mellitus ranged from 2.1% to 8.1% in South-East Asian countries. It was 2.0% in Indonesia, 2.3% in Thailand and 2.9% in India [5].

In our study, there were differences in prevalence of NCDs in plain and hilly areas as well as rural and urban populations. The prevalence of obesity and diabetes mellitus were higher in plain area while the prevalence of hypertension was higher in hilly area. This may be due to racial differences in these two areas. In this study, the majority are Bamar in plain area while Shan, Palaung, Kokant and other races are mainly living in hilly area. They were found to be different in personal habits, such as preference on sweet food, preference on exercise, alcohol consumption and smoking, and there may be more factors which we

have not been able to explain in this study and which may play a role in the difference in prevalence. The influence of racial differences in groups of study population upon the prevalence of NCDs was reported from study in Kayin State of Myanmar [2]. Moreover, the prevalence of hypertension, diabetes mellitus, and obesity was higher in urban area than in rural area. The higher prevalence of hypertension and diabetes mellitus among urban residents than rural residents was also reported in [6, 7, 8, 9].

In our study, the prevalence of diabetes mellitus was higher among females. The monotonic increment in the prevalence of diabetes mellitus was found with age. A study in India reported that overall prevalence of diabetes mellitus was increasing from 1% to 18% among males [10] and from 1% to 14% among females [11] between 1994 and 2003. The prevalence of diabetes mellitus increased with age was reported from studies in Kayseri [12] and United States [13].

In our study, the prevalence of hypertension and diabetes mellitus among obese were higher than among people with normal body weight. Similar finding was reported from many studies [14-16]. Our study revealed that there was a significant association between hypertension and diabetic patients. Many studies also reported increased risk of hypertension among the diabetic patients [17-19].

In our study, about one third of hypertensive patients reported that they had past history of hypertension and nearly one-fourth of diabetic patients had known themselves that they are diabetics. However, over two third of hypertensive patients and about three-fourth of diabetic patients did not take proper and adequate treatment.

In conclusion, the common NCDs in Myanmar including obesity, hypertension and type 2 diabetes mellitus were correlated to each other. The risk factors were multifactorial including non-modifiable e.g. ageing, gender, residential etc as well as modifiable e.g. smoking, alcohol con-

sumption, preference to sweet and salty diet, lack of exercise etc. Therefore, the people with non-modifiable characteristics should be regarded as target groups to give special attention. For people with modifiable risk factors, our study findings indicate that behavioral change communication program involving cessation or reduction of smoking, life style modification concerning preference to salty diet and sweet food, beneficial effects of exercise and encouraging to seek proper and effective treatment should be introduced through appropriate multimedia channels to reduce the risk of hypertension, diabetes mellitus and obesity as well as their complications.

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Molecular detection of human papillomavirus (HPV) genotypes associated with squamous intraepithelial lesions (SIL) and cervical cancer in Myanmar by polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) and sequencing method

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The aim of this study was to determine the human papillomavirus (HPV) genotypes associated with squamous intraepithelial lesions and cervical cancer in Myanmar by PCR-RFLP and sequencing method. A total of 145 formalin fixed paraffin embedded cervical tissue samples i.e 60 squamous intraepithelial lesions (SIL), 75 invasive squamous cell carcinoma (ISCC), 10 adenocarcinoma were collected from Pathology Department, Central Women Hospital, Yangon during 2007-2009 to find out HPV genotypes. Firstly, the diagnosis of those cervical tissues was confirmed by histopathology. Then DNA extraction was done. Two different consensus sequence primer pairs were used to amplify high-risk HPV-DNA (HPV-16,-18,-31,-33,-52b,-58) and low-risk HPV-DNA (HPV-6, -11). Multiplex PCR using two set of primer pairs within E7 & L1 translation regions were also used to detect HPV-DNA. Beta-globin primer was used for the detection of DNA integrity. The PCR product was confirmed by polyacrylamide gel electrophoresis with silver staining. Identification of the amplified product was confirmed by restriction fragment length polymorphism. Among 145 samples, 77 (53.10%) showed positive HPV-DNA using the PCR reactions with different primer pairs i.e 28 (46.67%) with SIL, 44 (58.67%) with ISCC and 5 (50%) of adenocarcinoma. Overall, the further genotyping with *Ava* II and *Rsa* I digestions revealed 83.67% of HPV-16, 4.08% of HPV-18, and 12.24% of HPV-31. There were only 2 positive benign HPV samples; one was HPV-6 and one unknown. Nucleotide sequence of HPV-31 was identified by cycle sequencing reaction. In this study, HPV-16 was the predominant genotype although other malignant HPV genotypes (HPV-18, -31) and one benign HPV genotype (HPV-6) were discovered out of 30 genital HPV genotypes.

INTRODUCTION

Globally cervical cancer remains an important cause of mortality among women, out of which 80% of women who present will have inoperable disease and around 300,000 will die each year [1]. It is the most common form of cancer in women in most developing countries and the second most common form of cancer in women in the world as a whole. In Myanmar, cervical

cancer constitutes approximately a quarter of new cases of female malignancies every year. The data from Yangon Cancer Registry reports over the past 30 years (1976-2006) showed that out of 56097 total commonest female cancers, cervical cancer accounts for 13181 cases. In terms of morbidity and mortality, cancer cervix has a reputation of always being either the commonest or the second commonest female cancers in Myanmar all along [2].

Human papillomavirus (HPV) plays a central role in the development of cervical cancer. The pathogenesis of cervical intra-epithelial neoplasia and cervical cancer is related to human papillomavirus (HPV) based on epidemiological, virological, and experimental evidences [3, 4].

Clinical and subclinical HPV infections are the most common sexually transmitted infections in the world [5]. Among asymptomatic women, the prevalence of genital HPV infections ranges from 2% to 44% [6, 7]. The age standardized prevalence rates of HPV infection in European, Asian, South American and sub-Saharan women without cytological abnormalities were estimated at 5.3%, 8.7%, 14.3%, and 25.6%, respectively, for a global estimate of 10.5% [8].

HPV are a group of small DNA papovaviruses. More than 100 types of HPV have been identified of which approximately 30 types are related to anogenital infections and can be passed from one person to another through sexual intercourse.

The forms of HPV infection may be latent, subclinical and clinical. HPV viruses also induce persistent infection in the genital tract carrying abnormal epithelial changes which in few cases could progress from low-grade dysplasia to cervical cancer. If a persistent infection with oncogenic HPV precedes virtually all high-grade dysplasias or neoplasias, thus the persistence for high-risk HPV-DNA is a marker for current or subsequent development of precursor lesions.

The most common "oncogenic" types of HPV are 16 and 18. HPV 16 is most often observed in invasive squamous carcinomas, whereas HPV 18 appears to have a predilection for lesions derived from the endocervical epithelium, such as small cell carcinomas and adenocarcinomas. Low-risk types such as HPV 6 and 11 can cause genital warts and a large proportion of low-grade squamous intraepithelial lesions of the cervix. HPV types 31, 45, 51, 52, 56, 58, 69 are commonly associated with CIN [9].

Several studies have indicated that viral persistence and the development of high-grade lesions and invasive carcinomas are closely associated with the presence of specific HPV variants. Given the different oncogenic potentials of the various HPV types and variants, HPV genotyping is becoming increasingly important as a diagnostic aid and prognostic tool. Recently, HPV detection has been proposed as an alternative to cytological screening for cervical cancer. Two main approaches are used for HPV detection and typing:

- (1) general primer PCR (GP-PCR) which amplifies a conserved region in the virus followed by type-specific identification, and,
- (2) the hybrid capture assay, which detects and types HPV by direct hybridization with type-specific RNA probes.

A variety of consensus primers are available for the detection of HPV. A variety of techniques, including DNA hybridization with type-specific probes, type-specific PCR, multiplex PCR with type-specific antisense primers, single stranded conformation polymorphism analysis, restriction fragment length polymorphism analysis and direct sequence analysis have been used in combination with GP-PCR to facilitate HPV typing [10].

Defining type-specific human papillomavirus (HPV) infections within cervical tissues is important for understanding the pathogenesis of cervical neoplasia and assessing the effectiveness of prophylactic vaccines with limited type-specific spectra. Because of different genotypes of HPV associated cervical neoplasia in different countries, it is very important to find out specific HPV genotypes associated with cervical neoplasia prevalent in Myanmar.

The aim of this study was to investigate the prevalence of human papillomavirus (HPV) infection associated with cervical neoplasia in Myanmar. The objectives were to find out the prevalence of human papillomavirus

(HPV) infection associated with squamous intraepithelial lesions and cervical cancer in Myanmar and to identify the HPV genotypes encountered with them in Myanmar.

MATERIALS AND METHODS

It was a laboratory-based descriptive study. A total of 145 formalin fixed paraffin embedded cervical tissue samples i.e., 60 squamous intraepithelial lesions and 85 cervical cancer were collected from Department of Pathology, Central Women Hospital, Yangon, Myanmar during 2007-2009. Some clinical and histological data of the patients were retrieved from the case files of Pathology Department, Central Women Hospital, Yangon and the findings were recorded in the proforma.

DNA extraction

A single 5-10µm section cut from the block was stained with hematoxylin and eosin to confirm the exact tissue analyzed. After histopathological confirmation, the subsequent sections from this block were placed in a 1.5 ml Eppendorph tube. After this tissue was treated with xylene and ethanol, it was suspended in the proteinase K solution at 50°C overnight. After centrifugation of the digested solution, the supernatant was treated with the mixture of 300 µl phenol and chloroform. The supernatant collected by centrifugation was added to 500 µl of isopropanol and stood in the freezer (-70°C) for 30 minutes. DNA precipitates were collected by centrifugation at 12000 rpm for 10 minutes and washed with 70% alcohol. Then DNA was dissolved in TE solution.

PCR reactions

For PCR reactions, two different consensus sequence primer pairs within the E6 and E7 open reading frames i.e., *pU-1M*, *pU-2R* and *pU-31B*, *pU-2R* were used to amplify malignant or high-risk HPV-DNA (HPV-16,-18,-31,-33,-52b,-58) and benign or low-risk HPV-DNA (HPV-6, -11), respectively. Multiplex PCR using two set of primer pairs

within E7 & L1 translation regions i.e., *OMY09*, *OMY11* and *OL1C1*, *OL1C2M* were also used to detect HPV-DNA. Beta-globin primer was used for the detection of DNA integrity. Reaction mixture was performed by using 0.15 µl of Ampli Taq Gold, 2 µl 10XBuffer, 3.2 µl dNTPs, 0.4 µl of forward and reverse primers, 12.85 µl distilled water and 1 µl DNA solutions. They are subjected to 35 cycles of amplification using Eppendorf Thermal Cycler. Each cycle included a denaturation, annealing and extension steps with different thermal conditions according to primer design. Detection of the PCR product was performed by the electrophoresis on a 6% polyacrylamide gel (PAGE) (19:1), 200V, 35-45 minutes and silver staining.

Genotyping of HPV by restriction fragment length polymorphism

High-risk HPV genotypes were decided by polyacrylamide gel electrophoresis - silver staining of the digest of PCR product with restriction enzyme(s), *Ava* II, *Rsa* I, *Bgl* II, *Acc* I and *Ava* I. Low-risk HPV genotypes were decided by using restriction enzyme, *Ava* II. The enzymatic digestion was done under the condition recommended by the manufacturer.

HPV-DNA sequencing by cycle sequencing reaction

PCR product was separated on a 3% NuSieve 3:1 in TEA solution at 70 volts for 60 minutes. After electrophoresis, the gel was stained with ethidium bromide and photographed under the ultraviolet light. The proper sized PCR product was cut from the gel, removed to a spin column and stood in freezer at -70°C for 30 minutes.

The extraction of DNA was collected by centrifugation at 12000 rpm for 10 minutes, and 2.5 times volume of isopropanol was added after addition of 0.1 times volume of 3M sodium acetate solution to the extract. Standing in freezer for 30 minutes, DNA precipitates were collected by centrifugation at 12000 rpm for 10 minutes, and washed with 70% ethanol. Cycle sequencing

reaction was performed by use of Big Dye Terminator Cycle Sequencing Kit (ABI). The nucleotide sequence was determined on a Gene Analyzer, ABI Prizm 310. The determination of genotype was performed by comparison with the nucleotide sequence of HPV released on the internet home page of NCBI.

Data analysis

Coded data obtained from the study were cleaned and entered into Microsoft Excel. After checking the range and consistency, prevalence of HPV in the study population was calculated.

RESULTS

DNA extraction

The chromatograms and the ratios between the absorbances at 260 nm and 280 nm of the DNA extracted by Proteinase K-Phenol-Chloroform method showed the rational pattern and the normal range and the concentration of their DNAs was enough PCR reaction (Fig. 1).

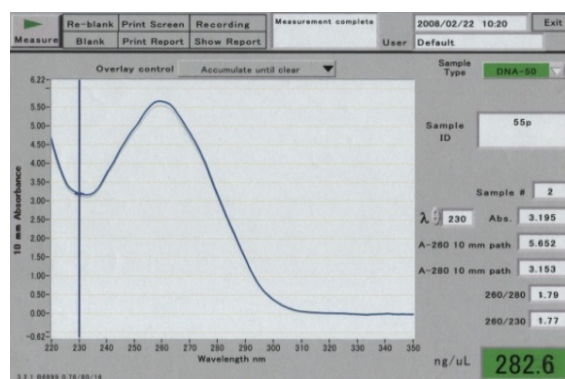


Fig. 1. Chromatogram of DNA solution

PCR reactions

With the PCR reactions using *pU-1M/pU-2R* primers, 49 samples (8 LSIL, 11 HSIL, 27 ISCC and 3 AdenoCA) revealed around 230bp band, showing the presence of the malignant HPV genotype, but with those using *pU-31B/pU-2R* primers, only two samples (1 LSIL & 1 HSIL) showed the positive result, revealing the band at around 230 bp (Fig. 2 a, b & Table 1). PCR reactions using multiplex primers (*OMY09*,

OMY11, *OL1C1*, and *OL1C2M*) showed the positive result in 26 samples (1 LSIL, 6 HSIL, 17 ISCC & 2 AdenoCa) of which 7 samples revealed two bands of 450 bp and 260 bp, 6 did one band of 450 bp and 13 only one band of 260 bp (Fig. 2 c, f).

Most of the samples (72.41%) were amplified with β -globin primer but about 28% did not react with it and, moreover, these also did not do with primer sets for the HPV-DNA. Additionally, although 72.41% of the total samples showed the positive PCR reaction with β -globin gene primer, only 53.10% of β -globin positive samples react with HPV primers but 19.31% did not react with any PCR primers for HPV (Fig. 2 d, e, f & Table 1).

Table 1. Prevalence of positive PCR for HPV-DNA by use of the different primer sets

Primers	SIL (n=60)			Cervical Cancer(n=85)			Total n=145 (%)
	LSIL n=30 (%)	HSIL n=30 (%)	Total n=60 (%)	ISCC n=75 (%)	Adeno -Ca n=10 (%)	Total n=85 (%)	
A	8 (26.66)	11 (36.67)	19 (31.67)	27 (36.00)	3 (30.00)	30 (35.29)	49 (33.79)
B	1 (3.33)	6 (20.00)	7 (11.66)	17 (22.67)	2 (20.00)	19 (22.35)	26 (17.93)
C	1 (3.33)	1 (3.33)	2 (3.33)	-	-	-	2 (1.38)
D	10 (33.33)	18 (60.00)	28 (46.67)	44 (58.67)	5 (50.00)	49 (57.64)	77 (53.1)
E	16 (53.33)	19 (63.33)	35 (58.33)	64 (85.33)	6 (60.00)	70 (82.35)	105 (72.41)

LSIL/HSIL= Low/high-grade squamous intraepithelial lesions

ISCC= Invasive squamous cell carcinoma

A= Positive PCR using malignant primers

B= Positive PCR using multiplex primers

C= Positive PCR using benign primers

D= Positive PCR using different primers

E= Positive PCR using β -globin primers

Digestion of the HPV-PCR product amplified with malignant primer set with *Ava II*

Among 49 samples having about 230 bp in size, 41 samples appeared two fragments, about 150 bp and 80 bp bands, being HPV-16 genotype and another 2 having about 270 bp in size appeared also two fragments, about 170 bp and 90 bp bands, being HPV-18

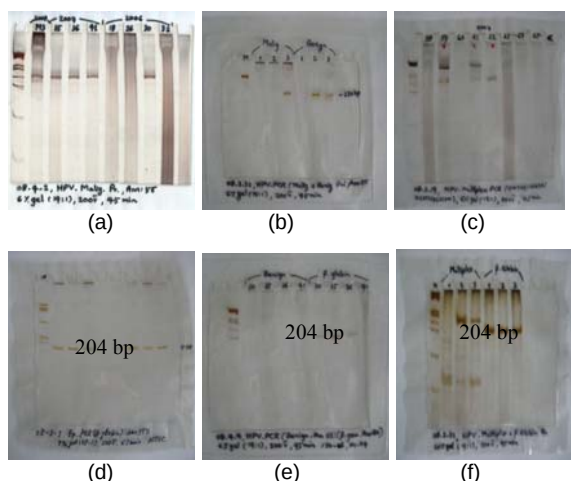


Fig. 2. (a) Photographs of the electrophoresis of PCR products amplified by use of malignant primer, (b) malig & benign primers, (c) multiplex primer (d) β -globin primer (e) benign & β -globin primers (f) multiplex & β -globin primers

genotype but 6 samples remained undigested with *Ava* II in the same original size (Fig. 3 a, b & Table 2). These samples were suspected of either HPV-31 or HPV-35 from the size of the PCR product. These should be further digested with *Rsa* I and moreover *Bgl* II.

Table 2. Malignant HPV genotypes decided by the digestion of PCR products with *Ava* II

HPV	SIL		Cervical cancer				Total (%)
	LSIL	HSIL	Total (%)	ISCC	Adeno Ca	Total (%)	
HPV-16	7	10	17 (89.47)	22	2	24 (80.00)	41 (83.67)
HPV-18		-	-	1	1	2 (6.67)	2 (4.08)
HPV-31 or 35	1	1	2 (10.53)	4	-	4 (13.33)	6 (12.24)
Total	8	11	19	27	3	30	49

Digestion of the HPV-PCR products amplified with benign primer set with *Ava* II

Two PCR products were detected in the PCR reaction with benign primer set in the samples of squamous intraepithelial lesions (SIL). They were digested with *Ava* II as described above. The digest had two fragment bands of about 130bp and about 100bp in size, being the genotype HPV-6 in one LSIL case (Fig. 3c).

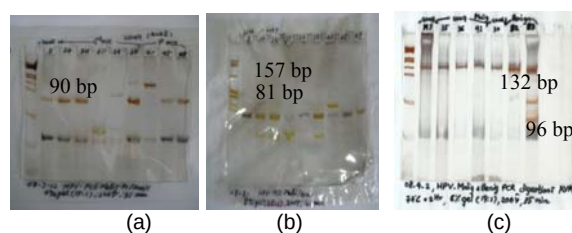


Fig.3. (a&b) Photographs of the electrophoresis of PCR product with malignant primers digested with *Ava* II (c) with benign primers digested with *Ava* II (6% PAGE (19:1) 200V for 35-45 min)

Digestion of the PCR product amplified with malignant primer set with *Rsa* I

The six samples in which the genotype of the PCR products obtained by the malignant primer set couldn't be identified by *Ava* II were further digested with the restriction enzyme *Rsa* I. These all samples having about 230 bp in size appeared the broad band overlapping the two fragments, about 119bp and 114bp regions, and thus they were decided as HPV-31 genotype (Fig. 4 a, b).

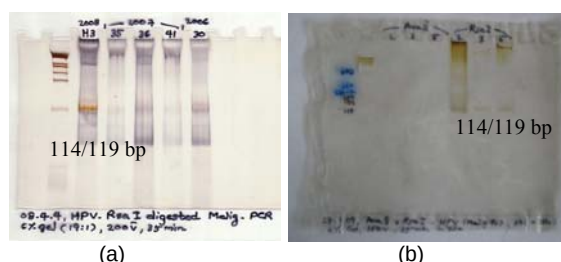


Fig. 4. (a, b) Photographs of the electrophoresis of PCR products with malignant primers digested with *Rsa* I, (6% PAGE (19:1) 200V for 35 min)

Sequencing of the PCR product amplified by use of malignant primer set and decided by the digestion with *Rsa* I

The nucleotide sequence of the PCR product (about 230bp) amplified by the malignant primer set was determined, although this was decided as the genotype HPV-31 by the digestion with *Ava* II following to the digestion with *Rsa* I. After the PCR product was purified by electrophoresis on Nusieve gel, the product was applied to cycle sequencing reaction following to a Sequence Analyzer. The result was compared

with sequence of the genotype HPV-16, and the consistency was 64%, showing that the nucleotide mutation was occurred in the various positions among the different HPV types. Although this product was already decided as HPV-31 genotype from the appearance of two fragment bands of about 115bp by *Rsa* I digestion, it was criticized from the presence of the restriction site of *Rsa* I enzyme in the center of the sequence of the sample.

DISCUSSION

Human papillomavirus (HPV) infection is now a well established cause of cervical cancer. HPV types 16 and 18 are responsible for about 70% of all cervical cancer cases worldwide. In South-Eastern Asia, about 6.2% of women in general populations are estimated to harbor cervical HPV infection at a given time, and 71.8% of invasive cervical cancers are attributed to HPVs 16 or 18 [11].

In this study, the presence of malignant HPV-DNA (16, 18, 31, 33, 35, 52b, 58) (high-risk types) and benign HPV-DNA (6, 11) (low-risk types) were detected using PCR-RFLP and sequencing method. Most of the samples (72.41%) were amplified with β -globin primer but 27.59% did not react with it and these also did not react with any primer sets for HPV-DNA. Additionally, although 72.41% of the total samples showed the positive PCR reaction with β -globin primer, only 53.10% of them reacted with HPV primers and 19.31% did not react with any PCR primers for HPV. Positive PCR reaction with β -globin primers is considered to be due to the presence of the human genomic DNA obviously shown to be extracted DNA from tissue blocks. Negative PCR reaction with β -globin primers might have some damages in DNA from paraffin embedded cervical tissue during formalin fixation, some inhibitors in the reactions, or some nucleotide mutations around the area to anneal with primers used in this study.

The PCR reactions using consensus HPV primers to detect the malignant or benign HPV types and multiplex PCR were not successful in all DNA samples. The samples being positive for the malignant primers might be expected to show the positive reaction, appearing at both 260 bp and 450 bp bands with multiplex primers, but the results were varied and appeared at 260 bp or 450 bp or both. The inconsistency in the PCR reactions between the malignant and multiplex primers was probably due to the HPV-DNA having many nucleotide mutations at the various positions even in the consensus primers.

Among 145 formalin fixed paraffin embedded cervical tissue samples i.e., 60 squamous intraepithelial lesions (SIL), 75 invasive squamous cell carcinoma (ISCC), and 10 adenocarcinoma, 77 samples (53.10% of the total) showed positive HPV-DNA using the PCR reactions with different primer pairs i.e., 28 (46.67%) SIL, 44 (58.67%) ISCC and 5 (50%) adenocarcinoma. With *pU-1M*, *pU-2R* primers, 49 (33.79%) revealed positive malignant HPV genotypes being 19 (31.67%) with SIL, 27 (36%) with ISCC, 3 (30%) of adenocarcinoma. Multiplex PCR reactions showed positive HPV-DNA in 26 (17.93%). Overall, further genotyping with *Ava* II and *Rsa* I digestions revealed 41 (83.67%) of HPV genotype 16 i.e., 17 (89.47%) of squamous intraepithelial lesions and 24 (80%) of cervical cancer. HPV genotype 18 was found in 2 cases (6.67%) of cervical cancer. HPV genotype 31 was seen in 6 cases (12.24%) being 2 (10.53%) of SIL and 4 (13.33%) of cervical cancer. There were only 2 positive benign HPV samples; one was HPV-6 and one unknown.

In this study, nucleotide sequence of HPV-31 was identified by cycle sequencing reaction. In matching with HPV 16 nucleotide sequence, the consistency of our sample sequence was not so high, but the presence of restriction enzyme *Rsa*I cleavage site (5'-GTAC-3') and the absence of *Ava* II site (5'-GGWCC-3') suggested the

HPV-31 positivity. Consensus PCR primers for the detection of HPV-DNA used in this study include one to five nucleotide replacements in the various positions of the nucleotide sequence. There was difficulty to designate the consensus primer matched to the different HPV genotypes which has many mutations at the various positions in the sequences of DNA. Thus, there may be the HPV-DNA that did not react with consensus primers although the HPV-DNA was extracted from the tissue. Even in this study, the DNA existed inconsistencies between the malignant and multiplex primers. Therefore, the primer design to detect different HPV genotypes should be considered further.

HPV type-specific primers have been used for amplification of HPV 6,11,16,18 and 33 nucleotide sequences but the number of primers necessary to amplify DNA specifically from every HPV type would make PCR impractical for the typing of clinical specimens [12, 13, 14]. Simultaneous detection and typing of genital human papillomavirus DNA was studied using the polymerase chain reaction with HPV consensus sequence primer pairs for the detection of different genotypes [15]. Identification of the amplified product was confirmed by restriction enzyme digestion. In this study, a *pU-1M/ pU-2R* mediated PCR was successfully applied to 39 cervical carcinoma samples; HPV-16 was detected in 19 cases, HPV-18 (5) cases, HPV-31 (2), HPV-33 (2), HPV-52b (1), HPV-58 (3) and unknown type (4) cases. Overall, the prevalence of HPV was 84.6%. This study indicated that this detection system may be useful for the detection of HPV not only of known types but also of new types.

The analysis described here does not need isotopes and is therefore convenient, especially where facilities to handle radioactivity are not available. The procedure is simpler than dot blot hybridization and can be performed within a day, although this system is less sensitive than a dot blot hybridization using isotopes. Additionally, it

would be useful for clinical diagnosis if benign HPV types (6, 11) and malignant HPV types (16, 18, 31, 33, 35, 52b, 58) could be amplified separately. Digestion of PCR products with restriction enzymes could identify HPV specific genotypes efficiently in clinical samples. In the same way, any combination of mixed infection could be identified because restriction fragments from each HPV type are of different sizes. But the decision of HPV genotypes by only PCR-RFLP method is sometime difficult since the various nucleotide replacements frequently occur in the sequence of HPV-DNA. It is considered that the sequencing method might be a better way for genotyping of HPV. This restriction enzyme analysis method followed by sequence analysis is particularly useful for identifying potentially new HPV types.

Conclusion

In Myanmar, the genotype of HPV-16 was the predominant genotype in our study although three different malignant HPV genotypes (HPV-16, -18, -31) and one benign HPV genotype (HPV-6) were discovered out of 30 genital HPV genotypes by the advanced molecular technique (PCR-RFLP-sequencing).

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